ANALYSIS OF PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH

1956

NATIONAL INSTITUTE OF
ALLERGY AND INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Annual Report of Program Activities

U. S. National Institute of Allergy and Infectious Diseases
January-December 1956

Extramural Program

From its inception in 1951 until July 1956, the grants program of this Institute was financed by annual appropriations of approximately two million dollars. The two and one-quarter million dollars allocated for the period July 1, 1955 to June 30, 1956 supported only 229 grants. By January 1, 1956 practically all of this fund had been expended and little remained to activate the 75 grants approved by the Council at the March meeting. The need for increased support of independent investigators working in selected fields of allergy and infectious diseases was again pointed out by the Advisory Council and the Study Sections as had been done in previous years.

The increased appropriation for fiscal year 1957 ($8,212,000) permitted for the first time a substantial expansion of this program. Beginning on July 1, 1956, progress was made on reducing the backlog remaining after the action of the March Council, in taking over active grants on infectious diseases previously supported by other Institutes because of lack of NIAID funds, and in activating grants approved by the Council at the June meeting. By the end of July, 70 per cent of the annual appropriation had been committed. Following action of the Council in November, the remaining funds were committed in activating approximately 75 per cent of the grants recommended for support at that meeting.

On December 31, 1956 there remained applications for approximately $600,000, which had been approved but which could not be activated because of lack of funds. In addition there were 19[illegible] grants for $2,600,000 pending action of the next Council meeting in March 1957.

In a field as broad as that encompassed by allergy and infectious diseases, there are a great many highly specialized areas frequently overlapping in interest and often competing for support. Particular attention therefore must be given to the maintenance of a properly balanced program. Recognizing that it is not possible to build up an effective program of research on specific diseases unless more general fundamental investigation is encouraged at the same time, projects which are of a very general scientific nature but which form the basis of the more specialized type of investigation are also supported.

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The approximate distribution of currently supported projects is:

Number	Major area	Amount
160	Bacteriology	$1,800,000
130	Allergy & immunology	1,800,000
110	Virology	1,700,000
120	Parasitology	1,100,000
60	Mycology	400,000
60	Arthropods	400,000
110	All others	1,000,000

Within these areas of broad support, research on vaccine development received special emphasis during this year. Including all projects and parts of projects which involve research basic and essential to the development of new and improved vaccines, approximately one million dollars were used for direct and indirect support in this field.

In support of research involving the use of germ-free animals, two grants for $22,000 have been activated this year and four additional ones for $190,000 are now awaiting action by the Advisory Council at its next meeting.

In late 1955 plans were made to expand the activities of this Institute in the fields of allergy and immunology. During 1956 particular attention was given to the implementation of these plans in the grants area. With the larger appropriation, it was possible to increase greatly the support previously given to independent investigators working in these areas.

During 1957 awards will continue to be made under the same rigid screening process as in the past. The continuing high growth potential for grant-supported research in allergy and infectious diseases is indicated by the large backlog of applications carried over from 1956 in spite of the relatively large appropriation which was available that year. The pattern of support in 1957 will in general follow that of 1956. If the requested increase is obtained, expansion will occur in three major areas:

(1) Immunology and Allergy. The need for encouraging research on specific aspects of allergy and immunology is well recognized. Of equal importance is the financing of supportive research related to these broad areas. These basic investigations are essential

to the production of sound data which will enable later investigators, conducting specifically oriented studies, to achieve results of practical significance. The application of such knowledge should eventually permit a direct attack, by means of either prevention or treatment, on specific diseases or allergic conditions.

(2) Virology. While substantial progress continues to be made in this field, the unprecedented expansion and rapidly accelerating developments necessitate constantly increasing support of research in this general area. During the course of recent investigations, many new and previously unrecognized viruses have been encountered. While this has clarified a number of previously confusing problems, additional ones have been created and new avenues of approach have been opened. It is imperative that advantage be taken of the opportunities thus presented and that immediate support be available for following up these promising leads as they develop.

(3) Tropical Medicine, including Parasitology and Mycology. These diseases continue to be of world-wide importance both economically and sociologically. The lack of satisfactory treatment for many of the parasitic diseases, the impact of arthropod-borne diseases (both parasitic and viral) upon the world's health, and the increasing importance of fungus diseases (which frequently produce serious, often fatal, generalized illnesses) constitute some of the current major problems in microbiology.

Accomplishment Highlights

A newly developed method of blood fractionation, plasmaphoresis, has been reported by grantees at Children's Hospital in Philadelphia. By this method, utilizing the Cohn blood fractionator, plasma has been collected 25 times from each of 23 donors in a period of one year. It is suggested that the procedure could be carried out as often as once weekly. This marked increase over the usual five donations a year is made possible by the action of the fractionator which separates the red blood cells and immediately returns them to the donor.

Grantees in Illinois reported that ultraviolet-irradiated poliomyelitis vaccine which was free of active virus was found to be capable of stimulating satisfactory levels of neutralizing antibodies to all three types of poliomyelitis virus in young children, a high percentage of whom had no detectable antibodies prior to vaccination. There was satisfactory persistence of antibody levels for at least one year. A booster dose of vaccine administered about a year after primary immunization effectively stimulated rises in antibody levels in those with low titers.

A grantee from Children's Hospital, Buffalo, New York, has reported studies on the occurence in man of a neutralizing antibody against canine distemper virus. The evidence so far available indicates that this neutralizing substance has the properties of a true antibody, formed in response to an as yet unidentified infection with an agent identical to or bearing an antigenic structure in common with canine distemper virus. The potential importance of this development is emphasized by the large number of American families having dogs as household pets and the increasing number of new and previously unrecognized viruses being encountered in the general population.

The latest findings from a continuing study on immunization against epidemic typhus has been reported by a grantee from Tulane University. A living rickettsial vaccine, utilizing a strain of Rickettsia prowazeki of greatly reduced virulence for man, has been given to approximately 17,000 individuals with no single known instance of truly serious illness resulting. Since the strain of Rickettsia used is apparently unable to invade the blood stream, it is unlikely that the organism could be picked up from an inoculated individual and spread by the louse vector. Available evidence indicates that immunity resulting from the use of this vaccine is solidly effective for at least three years and probably considerably longer.

A new approach toward solving basic problems in immunology is illustrated by the work of biochemists at the University of Wisconsin working under an NIAID grant. With various synthetic polypeptide compounds, they have been able to inhibit multiplication of mumps and influenza B viruses in chick embryos, and have demonstrated that some of these polypeptides and polypeptidyl proteins excite the formation of antibodies in rabbits. This finding could be the initial step toward the ultimate goal of a synthetic vaccine prepared from synthetic polypeptides.

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Clinical Investigation

This period marks the third year of the clinical investigation of infectious diseases using the facilities of the Clinical Center. During this time new programs have been developed and established programs in the Institute have been expanded and directed toward clinical aspects of the diseases caused by microbial agents. The full complement of beds assigned to the Institute has now been activated allowing sufficient facilities to hospitalize patients for a variety of clinical studies. The staff has been enlarged to take care of the increased number of patients but this has been largely by the addition of younger physicians who, though well trained in clinical medicine, have not been trained in clinical research nor had long experience in clinical judgment.

The year has seen a continuing reorientation of efforts directed toward the investigation of clinical problems which can be studied advantageously. These have been largely those related to long term infectious disease or diseases which are within the interest of some section of the Institute. In general, the acute infections with the exception of respiratory infections due to viruses have not been included in the research programs. This has resulted in the emphasis being placed on such diseases as hypogammaglobulinemia, mycotic infections, amoebic and helminthic infections, sarcoidosis, and cystic fibrosis of the pancreas with its attendant bacterial infections.

As a measure to strengthen the program general guidelines have been formulated. These include plans for investigations in broader and more fundamental clinical fields, concentration on fewer research projects, development of improved methods of case selection and referral, and provisions designed to aid investigators in their work, such as certain periods for full-time laboratory work, encouragement of cooperation with non-clinical research units, and repeated effort to recruit qualified clinical investigators in pediatrics. In view of the increasing emphasis on research in immunology and allergy, the immunologic aspects of infectious diseases were pursued from a clinical standpoint, and clinicians encouraged to seek the cooperation of the increasing number of scientists working in this area in the Institute.

In studies on acute respiratory diseases, the newer knowledge of the adenovirus group has been applied to the identification of specific infections and a total of 145 patients, both those admitted as clinical patients and observed as out patients, have been identified as being infected with this group of agents. The clinical characteristics of persons suffering from these infections have been carefully observed and described by the intensive studies made possible by the facilities of the Clinical Center. A method for the recognition of mumps virus in cerebrospinal fluid and urine by multiplication of the virus in tissue culture has been developed and a total of 17 patients studied with this technique.

Systemic fungal disease, including cryptococcosis, nocardiosis, coccidiodomycosis, aspergillosis, blastomycosis, and histoplasmosis, has been observed and the opportunities taken to study such facets of infection as adrenal, liver, pancreatic, and brain involvement. Of major interest has been the response of infection to newer drugs, including endomycin and Amphotericin B. These studies in patients have been consistently supported by laboratory investigations in conjunction with the Mycology Unit of the Laboratory of Infectious Diseases. Certain of the mycotic infections, such as histoplasmosis, are being recognized more frequently. Others, such as moniliasis, create problems by the ubiquity of the organism which may grow more abundantly and cause systemic disease in the presence of most antibiotics and steroids, creating serious and extensive clinical problems. The studies here have been an unusual opportunity and a good demonstration of the value of a broad attack upon infections which are notoriously difficult to solve.

One of the challenging problems has been the etiology and pathogenesis of aseptic meningitis. Ordinarily, this clinical condition is related to a causative agent in only a small proportion of cases. The clinical experience with twenty patients here has revealed the apparent etiologic cause in nineteen of them. This is an unusually high percentage which reflects adequate and complete examination of many specimens and proper handling and preservation of them. The Coxsackie group of viruses has been most frequently recovered and a group of patients infected with this agent studied carefully to characterize the spectrum of clinical illness.

In cooperation with Howard University, students of that school who have lived outside the United States were studied for the presence of filariasis. This casefinding method afforded the opportunity of careful study of a number of individuals and showed that up to 10 per cent of foreign students from endemic areas are infected with this parasite. Similarly, State Department employees returning to the United States are examined for the presence of other parasitic infections, including amoebiasis and helminthiasis. Appropriately selected patients are admitted to the Clinical Center where diagnostic and therapeutic evaluations can be undertaken. These studies are closely collaborative with those being undertaken with the Laboratory of Tropical Diseases.

Trichomoniasis, a disease of increasing medical interest, was investigated epidemiologically and therapeutic regime evaluated. Its prevalence and the unsatisfactory results of treatment emphasize the need for continued research in all phases of the disease.

Definitive clinical information not reported previously was secured on two cases of laboratory proved toxoplasmosis. This included incubation period, clinical manifestation and course, serological response and changes, and response to pyrimethamine therapy.

One of the most productive of the investigations has been that concerned with hypogammaglobulinemia. These studies have developed methods of determining the persistence of antibody in patients in terms of

half-life (days) and show that antibodies may vary in this regard. This study is a good example of the use of clinical material in basic immunologic research.

The studies on cystic fibrosis of the pancreas in cooperation with the National Institute of Arthritis and Metabolic Diseases illustrates the role of bacterial infection in an essentially metabolic disease. A number of children with this disease have been admitted to the Clinical Center and the bacterial flora of the sputum investigated. Some new observations are that the strains of staphylococci, the most common infecting organism of the lungs, remain relatively constant and are frequently not resistant to the antibiotic that the patient is receiving. The fact that this disease may occur in as many as one in a thousand live births and that it is frequently overlooked renders it of more importance than has hitherto been considered.

A number of other diseases are investigated clinically when patients are available for admission. These include brucellosis, streptococcal infections, enteric bacterial infections, poliomyelitis and influenza which afford clinical material and questions for ongoing laboratory studies in the Institute. A sustained effort is made to bring laboratory investigators to the clinical problems of the bedside and to offer opportunities for clinical observations. In turn the clinical investigators freely call on scientists with pertinent information or skills for collaboration in the pursuit of research. This collaborative effort also extends to the universities and medical schools in this vicinity.

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Laboratory Research

Plans for establishment of a concerted research program in immunology formed a major feature of the Institute's activity during the year. The increasing interest and responsibility for advancing knowledge in basic immunology, hypersensitivity phenomenon, and allergy, as reflected in the recent change of Institute name, were implemented by plans to coordinate existing intramural investigations in these fields and to supplement them with new studies. The beginning of this permanent program is being made by concentration on the laboratory and basic aspects of immunology with the intention to expand into clinical investigation as promising leads develop, investigators conceive original projects, and facilities become available. The Institute invited an immunologist of world-wide reputation to become a consultant to this program in order to assist in selection of the staff and to give coordinated direction to research projects. He subsequently accepted a permanent staff position to assume responsibility for the newly created Laboratory of Immunology as its first Chief, effective January 2, 1957. Establishment of this Laboratory represents a major advance in a research area which has great need for intensive study from the viewpoint of both clinical medicine and basic science.

The year also marked the initiation of several other programs representing an entrance into new areas of infectious disease research. One of these is that on germ-free animals (gnotobiotics). In collaboration with the Sanitary Engineering Branch of the Division of Research Services and based upon the pioneering work at LOBUND Laboratories at the University of Notre Dame, three tanks have been set up in which germ-free guinea pigs were delivered by Caesarian section and are being reared. As experience is gained with these specialized procedures, the animals will be used in studies on trichomoniasis and the extension of the studies on amoebiasis by an Institute staff member at LOBUND. Availability of this new tool for research promises to stimulate a number of productive investigations in infectious diseases and with scientists working in other institutes in non-infectious diseases.

Studies on the physico-chemical methods for the isolation of fractions have been intensified because of the promising results arising out of preliminary work in the past. Certain bacteria, including Salmonella, Mycobacterium, and the fungus Histoplasma, have been fractionated by physical means and the process observed by electron microscopy and the fractions characterized by chemical, bacteriological, and physical methods. This procedure resulted in separation of the cell wall from protoplasmic material and permitted the demonstration in some bacteria that protective immunity in experimental animals is produced by injection of cell walls which possess low toxicity.

As a continuation of the studies on the viral therapy of cancer, efforts during the past year were extended to clinical studies in collaboration with the National Cancer Institute. Some thirty cases of cervical carcinoma were injected with oncolytic adenoviruses and the clinical course of the patients observed. While there was local destruction of the tumor, no permanent therapeutic effect was obtained. Stimulated by this work, a new technique for growing human cancer cells in the peritoneum of irradiated rats was developed, promising a laboratory tool for the evaluation of oncolysis by this virus. This method is currently being evaluated and strains of Coxsackie virus selected by this method are to be used in human clinical cases.

The increasing concern of the Public Health Service with diseases occurring in the Caribbean area, particularly the northward march of yellow fever as far as Guatemala, has focused attention upon the research needs in this field. Plans are under way for an expanded program to investigate such diseases and a small beginning has been made by diverting personnel and facilities toward this end. Two members of the staff have made a field trip into Guatemala and a species of Central American mosquito has been colonized in the insectary at Bethesda. The scientific interests of the Institute will be utilized in support and extension of this responsibility in the future. Current thinking contemplates a long range research effort probably located in the Canal Zone and cooperating with the other branches of the Government which have interests in this area, particularly the military.

Contributions to knowledge and understanding of viruses and diseases caused by them continued to form a major share of the productive work of the Institute of Allergy and Infectious Diseases. A group of the respiratory viruses recently named the adenoviruses has received particular attention again this year and much new data bearing on their role in acute respiratory disease revealed.

Additional evidence was acquired confirming the role of certain adenoviruses in acute respiratory disease in naval recruits, in pharyngoconjunctival fever in civilian populations, in follicular conjunctivitis and in undifferentiated respiratory disease in infancy and early childhood. Intensive study of the viral infections occurring in an orphanage nursery population revealed high infection rates with adenoviruses types 1, 2, 3, and 5. These studies, as well as additional serological surveys, show that these four virus types are the most frequent adenovirus infections of man, and emphasize the fact that the vast majority of experiences with the adenoviruses occur during infancy and early childhood. Interpretation of their role in extremely frequent febrile illnesses observed in the orphanage is complicated by frequent bacterial infections as well as infections of the enteric agents such as Coxsackie and the ECHO viruses.

Preliminary to commercial preparation of polyvalent or multivalent adenovirus vaccines, it was necessary to adapt adenovirus types 1 through 7, directly from human sources to monkey kidney cells. Following this, collaborative studies with the NAMRU #4 at the Great Lakes Naval Training Station showed in military recruits that a trivalent vaccine (containing types 3, 4, and 7) apparently prevented 60 per cent or more of the ARD and ARD-pneumonia illnesses expected. This study included more than 16,000 recruits, of which 4,000 were vaccinated and 12,000 were retained as controls.

Studies of adenovirus and other respiratory viruses in several hundred reformatory volunteers, in collaboration with Dr. Thomas Ward of the LOBUND Institute (formerly at Johns Hopkins School of Hygiene), confirmed and emphasized similar information acquired in studies of natural disease. The role of local irritation in establishing adenovirus infection of the conjunctiva followed by objective evidence of illness, was described and was utilized in demonstrating the human pathogenicity of adenovirus types 1, 3, 4, 5, and 7. Each of these agents produced an objective syndrome similar to pharyngoconjunctival fever.

Laboratory methods for investigating an interesting and difficult agent, the salivary gland virus, were developed, and when applied in large scale serological studies showed the salivary gland virus (cytomegalic inclusion disease virus) to be a highly prevalent infectious agent in man. These data increase the possible importance of this latent agent in various chronic diseases in children and the new serologic methods provide the tool for investigating the matter.

Essential to cell culture of viruses is knowledge of cellular biology and nutrition. Studies of the nutrition of mammalian cells in tissue culture revealed further progress in defining the metabolic requirements of these cells. For instance, myoinosotol was found to be an essential factor in a defined tissue culture medium. Similarly, glutamine was found to be an essential building block for synthesis of protein, and in other studies, along with glucose and salts, was found to provide essential nutritional factors for the growth of poliomyelitis virus in HeLa cell tissue cultures. Interestingly enough, it was found that the carbohydrate utilization was not different in normal and malignant cells. The availability of cancer cells grown with defined media in tissue culture, led to rather extensive studies of the effects of a variety of anti-tumor chemicals on such cells. Cytotoxicity of a number of anti-tumor chemicals was found to be so high as to suggest that this activity may be quantitatively related to their anti-tumor action in vitro.

Of particular interest was the crystallization and the fractionation by hydrolysis of a Coxsackie A virus, representing the first crystallization of a representative of this group of viruses and, actually only the second report of successful crystallization of a mammalian virus. Other noteworthy contributions included the

specific identification of the Russian polio virus type 4 as Coxsackie virus type A-7 and the determination of certain nutritional requirements of poliomyelitis virus grown in HeLa cells.

As an outgrowth of the participation in the poliomyelitis vaccine evaluation of 1954 and investigation of the "Cutter incident" in Idaho in 1955 Institute scientists found much information of value.

The present monkey safety test employed for the release of poliomyelitis vaccine resulted from the attempts made to isolate poliovirus from Cutter vaccine. The epidemiology of poliomyelitis induced by injection of live virus into children was studied. The primary cases were indeed severe, but the degree of illness among secondary cases was not greater than occurs under usual circumstances, and the number of secondary cases was less than expected. In connection with the vaccine program, vaccination of children with commercial lots of poliomyelitis vaccine was done. The results indicated that the vaccines available were less potent than those employed in the field trials and emphasized the need for a complete course of immunization. It was also shown that an antigen is shared jointly by types 1 and 2 poliovirus, but that the possession of type 3 antibodies did not influence production of antibodies against type 3 virus. Type 3 virus apparently shares an antigen with type 2 but not type 1 virus. Thus, in areas where the incidence of natural diseases is high one might expect a more favorable response to immunization than in those areas where the incidence of disease is low, since there would be less chance for cross stimulation to occur.

Colorado tick fever continues to be a problem in the western United States, and there are increasing numbers of reports of severe illness induced by infection with this virus. These severe cases take the form of hemorrhagic manifestations or of encephalitis. The virus has been isolated from nymphs of Dermacentor andersoni as well as from adult ticks of this species.

It was previously reported that in Culex tarsalis the extrinsic incubation period of western equine encephalitis virus was about 4 days and that donor animals must have a viremia with a titer of 10^4 virus particles in order to infect an appreciable number of mosquitoes. Investigations of a like nature carried out with St. Louis encephalitis virus indicate that a similar titer of virus is needed to infect mosquitoes, the extrinsic incubation period is at least 8 days, and that the temperature at which C. tarsalis is maintained greatly influences the titer of virus in the mosquitoes and their ability to transmit infection. These observations showing that St. Louis virus requires a longer time and a higher temperature

to develop in mosquitoes than does western equine encephalitis virus probably account for the comparatively low incidence of St. Louis virus infections in the Northwest. Other studies with insect vectors demonstrate that certain soft-shelled ticks are capable of maintaining virus following ingestion of infected blood.

The community health project in Montana has continued to function in determining the effect of febrile diseases upon the general health of a community. A serological study of residents of Hamilton shows that antibodies against Coxsackie viruses have essentially the same age distribution as do those against poliovirus. Continued studies of the degree of contamination of the sewage system by viral agents demonstrate that adenovirus and ECHO viruses are common from such a source. By a new technique employing multipore filters to obtain samples from raw sewage for tissue cultures, a larger number of positive results are obtained, the incidence of contaminated cultures is reduced, and the time and labor of obtaining specimens is decreased considerably. The use of a variety of cells in a battery of tissue cultures has improved the rate of recovery of cytopathogenic agents.

A concerted program in the field of immunology revolves upon the use of physico-chemical methods for the isolation of chemical and morphological fractions responsible for the production of immune reactions of various types in experimental hosts. During the current year, much work has been done to determine the relation of toxicity to antigenicity, the nature of Boivin antigens, and the production of protective immunity against Salmonella enteritidis infections in mice. There is little protection afforded mice by immunization with protoplasmic fractions. Protection is produced, however, by injection of cell walls with no regard to their toxicity. Separation of toxin and immunizing antigen from cell walls by extraction with ether demonstrates that toxin affords no protection against infection and that the specific antigen is essentially nontoxic. The Boivin antigen appears to be an artifact induced by the severe chemical treatment employed for isolation.

Preliminary experimentation with acid-fast bacilli was done, and it was found possible to obtain cell walls from Mycobacterium butyricum by physical methods. It was necessary to add a detergent to the suspension of organisms during treatment in the mechanical disintegrator to prevent clumping and this, incidentally, made it easy to separate the protoplasm from the cell wall since the former was soluble in the medium. Cell walls have been obtained from the BCG bacillus and from mammalian cells grown in tissue culture. Monkey-kidney cells did not yield cell walls because of their fragility but HeLa cells and conjunctival cells are sturdy and, from these, cell walls and nuclear walls have been obtained.

In addition to the establishment of germ-free animal research at Bethesda, the work at LOBUND Laboratories was continued. Previous efforts to produce amoebiasis in germ-free guinea pigs met with failure unless the animals were fed concommitently with single species of bacteria, such as Escherichia coli or Aerobacter aerogenes. At the same time, conventional animals showed in nearly every instance symptoms and lesions of acute amoebic dysentery, from which most of them succumbed. More recent studies indicate that under certain circumstances Entamoeba histolytica may become established and produce lesions in the intestinal tract of germ-free guinea pigs. Introduction of certain Seitz filtrates of fecal suspensions from conventional guinea pigs enabled the amoebae to become established. Also it was found that by varying the OR potentials of the gut to a certain extent the amoebae were able to survive and produce lesions.

The pilot control project for schistosomiasis in the Waraq el-Arab area near Cairo, Egypt, in cooperation with the International Cooperation Administration and the Egyptian Ministry of Health, was turned over to the latter organization on July 1, 1956. During the three years in which personnel from this laboratory directed this project, transmission of the disease within the area was entirely prevented by the use of one method of control alone, i.e., the application of the molluscacide sodium pentachlorophenate. During the first two years there was a substantial reduction in the incidence of infection in school children ages 6 to 10 years. A report for the third year of operation is not available because of the political situation in the Middle East, but it is anticipated that additional reduction has taken place.

In connection with the schistosomiasis control demonstration project in Brazil, in cooperation with the Brazilian Ministry of Health and the Pan American Sanitary Bureau, control programs were inaugurated or continued during the year in various areas in the States of Pernambuco, Bahia, Belo Horizonte, Ceara, Para, and Espirito Santo. As an example of the progress of this work, it may be mentioned that the disease has been definitely under control for over a year in 250 communities in the State of Bahia, with protection of approximately 300,000 people at an operational cost of about $0.08 per person. With a few possible exceptions, no snail intermediate hosts of schistosomiasis have been found in the area during the past year. Laboratory participation in this program was discontinued as of December 31, 1956, when the Brazilian Ministry of Health assumed supervision of the control campaign.

In basic studies on drug resistance, chicks infected with Plasmodium gallinaceum were employed. Resistance to the drug pyrimethamine developed and persisted through mosquito passages of

the parasite. During the year, it was shown that malaria strain hybrids can be produced, that these hybrid strains are also resistant to the drug, and that this resistance can be transferred through the mosquito vector.

In a study financed by the World Health Organization, inmate volunteers were given anti-malarial drugs in a salt mixture, with the view that such administration in isolated areas, in which other methods of malaria control might not be feasible, might result advantageously. Pyrimethamine at a calculated dosage of 25 mgm. per week and chloroquine at a calculated dosage of 300 mgm. of the base per week each produced complete suppression of vivax malaria without evidence of toxicity when administered in such a salt mixture.

Previous work in this laboratory had indicated the value of a new antibiotic, Stylomycin, against Trypanosoma gambiense, one of species responsible for African sleeping sickness. Clinical trials in Portuguese Guinea indicated its value in human patients. Further studies have shown that another antibiotic, Nucleocidin, is effective against T. gambiense and also T. congolense, a parasite of cattle, as well as T. equinum, a species found in equines. These two antibiotics are the first to have demonstrated value against any trypanosome infection.

During extensive investigations around the slum-area premises where a fatal congenital case of toxoplasmosis presumably was contracted, Toxoplasma infection was found to be enzootic in the animal population of the immediate neighborhood. Parasites were recovered frequently from cats, mice, dogs, pigeons, ducks, and chickens. The infection was found in 37 per cent of the mice trapped in the home of the patient.

A new hemagglutination test for toxoplasmosis has been developed. This test appears reliable and specific and offers results comparable to the Sabin-Feldman dye test. In further studies on granulomatous uveitis, an analysis of serological test results indicates that at least 35 per cent of such cases may be due to Toxoplasma gondii. In this connection, it was found that experimental ocular toxoplasmosis in rabbits could be controlled by a dosage of 100 mgm./kg/day of pyrimethamine or diaminodiphenyl sulfone. Tissue culture tests indicate that the former drug is stored in the host cells.

Leptospiral antigen has been localized in the livers of infected animals by the fluorescent antibody technique. The antigen was of a particulate nature and involved principally the cytoplasm of certain cells. Furthermore, the leptospiras were localized and observed to lie as wreaths around the parenchymal cells in the intercellular spaces. It has been shown that tissues frozen by the conventional techniques show many ice crystal artifacts and must be frozen rapidly in petroleum ether at -70°C or isopentane at -195°C. The discovery was made that much greater fluorescence was effected by exciting the labelled tissues at a wave length of 410 mu rather than 365 mu.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 1
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Office of the Director
LABORATORY, BRANCH, OR DEPARTMENT

4. none
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Office of the Director
PROJECT TITLE

7. Dr. Victor H. Haas
PRINCIPAL INVESTIGATOR

8. none
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION:

During the current year the development of new and expanded activities and research programs have been of major concern. Through the establishment of a new position, that of the Associate Director, responsibility for coordination, planning and general direction of the intra-mural research of the Institute has been consolidated.

The first full year of the NIAID grants program under a separate Council has been completed. Considerable progress has been made in securing more adequate support for basic medical research in microbiology.

Germ-free laboratory facilities have been developed to the stage that NIAID staff has completed the first experimental tests in production of animals. In organizing the newly created Laboratory of Immunology, research projects are being modified and coordinated where possible to utilize existing resources.

Plans for the new Laboratory of Immunology have progressed to the extent that a chief has been appointed, and the staffing is underway. Adjustments of existing space and facilities and consolidation of research related primarily to immunology is progressing.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-1
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	75,000	37,498	112,498	4.0	5.5	9.5

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	4	7	11	

13. BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	/ ☒
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID - 1
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Nadel, E. and Haas, V. H. - Effect of the virus of lymphocytic choriomeningitis on the course of leukemia in guinea pigs and mice. J. Natl. Cancer Inst., Vol. 17, 221-231, 1956.

Stewart, S. E. and Haas, V. H. - Lymphocytic choriomeningitis virus in mouse neoplasms. J. Natl. Cancer Inst., Vol. 17, 233-244, 1956.

Haas, V. H. and Stewart, S. E. - Sparing effect of a-methopterin and guanazolo in mice infected with virus of lymphocytic choriomeningitis. Virology, Vol. 2, 511-515, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1955

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORTS

Part A. Project Description Sheet

1. NIAID - 2
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Office of the Scientific Director
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Scientific Direction and Planning
PROJECT TITLE

7. Dr. Dorland J. Davis
PRINCIPAL INVESTIGATOR

8. none
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

This is an activity which, prior to this year, was conducted as a part of the administrative functions of the NIAID which came under the immediate direction of the Director of the Institute. The large increases in intramural research activitites of the Institute resulting in new problems of program planning, coordination and development created a need for more direct control and assistance.

The objectives of this unit are: (1) to determine where the need exists and to direct adjustment of resources of the Institute to meet changing conditions of scientific progress and development to anticipate the direction and extent of medical research requirements of the future.

(2) to council and advise on specific research problems and to coordinate and consolidate the research programs of the Institute in a manner designed to obtain maximum effectiveness; (3) to assist in the development of research activitits of the Institute along lines which will encourage productive cooperation between segments of the Public Health Service, the Department and other Federal agencies; (4) to evaluate the scientific output of the Institute and to assist in the dissemination of research findings to meet whatever legitimate needs exist through the appropriate means of communication.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 2
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	40,000	20,192	60,192	1	1	2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	

13. BUDGET ACTIVITY:

RESEARCH [X]
REVIEW & APPROVAL [ ]
BIOLOGIC STANDARDS [ ]
ADMINISTRATION [ ]
PROFESSIONAL & TECHNICAL ASSISTANCE [ ]

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards and Publications

15. NIAID - 2
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Davis, D. J. - Occurrence of Influenza July 1955 to June 1956. Public Health Reports, Vol. 71, No. 12, 1225-1227, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 3
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Office of the Director
LABORATORY, BRANCH, OR DEPARTMENT

4. none
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Research Auxiliary Section
PROJECT TITLE

7. Kenneth H. Brown
PRINCIPAL INVESTIGATOR

8. Earl B. Dillehay
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

none

10. PROJECT DESCRIPTION:

The purpose of the Research Auxiliary Section is to provide central services which cannot be effectively performed by the individual laboratories. The work of this group includes supplying the laboratories with animal food, bedding, dry ice; cleaning animal rooms and cages; operating the building incinerators; providing central cage sterilization; continuing over weekends the feeding and observation of animals; collect dirty glassware from the laboratories, replacing it with sterile glass supply, maintained in the central self-service, glass stock room.

Without increasing personnel during the current year this unit has serviced the expanded laboratory operations of the Division of Biologic Standards. This has been possible largely as a result of the changes in animal diet and feeding schedules which permitted savings in time of food preparation and distribution.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 3
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	42,100	**	42,100	-	8.0	8.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	-	13	13	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

**Reimbursement cost of $10,500 spreads to laboratories.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID - 3
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-4
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Review and Approval
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Extramural Program
PROJECT TITLE

7. Dr. Leonard Karel
PRINCIPAL INVESTIGATOR

8. Dr. Morris T. Jones
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RECEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

I RESEARCH GRANTS REVIEWED DURING CALENDAR YEAR 1956

	Received		Approved	
March	No.	Amount	No.	Amount
New	83	951,248	62	626,489
Continuations	13	110,783	13	110,783
	96	1,062,031	75	737,272

(Use reverse and addtional pages, if necessary)

I RESEARCH GRANTS REVIEWED DURING CALENDAR YEAR 1956 (Continued)

June	Received No.	Received Amount	Approved No.	Approved Amount
New	225	2,743,546	187	2,271,853
Continuations	171	1,975,553	124	1,525,425
Transfers from other Institutes	---	---	119	1,232,664
	396	4,719,099	430	5,029,942
November				
New	236	2,936,208	189	2,172,222
Continuations	47	450,128	47	450,128
	283	3,386,336	236	2,622,350

By December 31, 1956 the appropriation of $8,212,000 for Fiscal Year 1957 had been committed.

II FELLOWSHIPS (Fiscal Year 1957 appropriation $117,000)

Fellowship applications considered by the NIAID Board ------ 135

Fellowship applications approved by the NIAID Board -------- 132

Awarded by other Institutes ------------------------------ 20

Awarded by NIAID --- 38

By Types

pre-doctoral ---------- 26
post-doctoral ---------- 10
special ---------- 2

By Catagories

Allergy ---------- 5
Antibodies ---------- 3
Arthropods ---------- 1
Bacteriology ---------- 6
Biochemistry ---------- 5
Genetics ---------- 1
Helminthelogy ---------- 2
Immunology ---------- 2
Mycology ---------- 3
Protozology ---------- 4
Virology ---------- 6

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-4
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$95,000	96,000	$191,000	4	7	11

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3	8	11	

13. BUDGET ACTIVITY:

RESEARCH	[ ]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[X]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Division of Research Grants.

(Use reverse and additional pages, if necessary)

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID-4
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

(Use reverse side and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 6 (c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Office of the Chief
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION:

Objectives:

To develop, direct and coordinate the program of the Laboratory, as defined in the individual research projects; where necessary, to re-direct individual projects to meet current needs and advances in the field; to align clinical projects with patient availability; to provide an unexcelled standard of patient care for patients utilized in research.

Methods Employed:

Organize available staff and recruit qualified personnel, both professional and sub-professional, to develop the program and carry out its aims; close liaison with area medical societies, institutions and individuals for referral of patients whose diagnoses fall within the active or proposed disease research categories; the highest standards of patient care are maintained by selection of qualified physicians; the development of necessary policies and continued close and direct supervision of patient care activities; professional consultant services to other institutes in the area of infectious diseases; continued guidance of research projects undertaken by younger investigators; maintenance of staff morale.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-6(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$30,459	$8,147	$38,606	1.0	4.0	5.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3	4	7	78

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID - 6 (c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 7(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Rheumatic Fever and Streptococcal Infection
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. Dr. Norman B. McCullough and Dr. James L. German
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases.

10. PROJECT DESCRIPTION:

A. Rheumatic Fever

Objectives:

To evaluate and establish clinical and laboratory criteria for diagnosis. To study pathogenesis of the disease, particularly the factors that lead to cardiac involvement, and experimental therapy.

Methods Employed:

Clinical studies. Evaluation of various fractions of streptococci and their corresponding antibodies as immunologic aids in diagnosis. Correlation of various biochemical tests with clinical observations.

Major Findings:

Series too small for evaluation.

Significance to Microbiological Research:

Rheumatic fever is a major unsolved public health problem. Aids to definitive diagnosis are badly needed.

Proposed Course of Project:

This project will be pursued if and when patient material becomes available.

B. Streptococcal Infection

Objectives:

To investigate the pathogenesis of acute diffuse glomerulonephritis. To determine the course and sequellae of this disease in children. To establish guidelines for therapy. To establish guidelines for the antibody therapy of subacute bacterial endocarditis.

Methods Employed:

Glomerulonephritis Clinical Studies: Immunologic studies employing various streptococcal derivatives in standard laboratory tests; the application of tissue culture techniques to cell-antigen systems possibly operative in this disease; determination of antikidney-protein antibodies in serum of patients; and correlation of results with history and clinical course.

Subacute Bacterial Endocarditis: Standard laboratory tests for determining antibiotic sensitivities of causative organisms; determination of antibiotic serum levels; use of various antibiotic regimens.

Major Findings:

Series is too small for definite conclusions.

Significance to Microbiological Research:

The pathogenesis of glomerulonephritis is unknown. The disease is common and sequellae are serious. The disease lends itself to the study of basic immunologic phenomena.

Proposed Course of Project:

Dependent upon availability of clinical material.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-7(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$8,470	103,193	111,663	0.7	-	0.7

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	-	1	980

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAI - 7(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Cramblett, Henry G.: Juvenile Rheumatoid Arthritis - A Review of the Literature. Clinical Proceedings of the Children's Hospital; April, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 8(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Respiratory Viral Diseases
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. Dr. Henry G. Chamblett and Dr. Thomas F. Dolan
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases.

10. PROJECT DESCRIPTION:

Objectives:

(a) To define clinical entities in relation to newly isolated respiratory viruses.

(b) Further to define diagnostic criteria, pathogenesis, immune response, persistence and sites of virus and effects on tissues of virus in certain infections of respiratory passages and mouth.

(c) To study host-parasite relationships in reference to susceptibility to chronic or recurrent respiratory diseases.

(d) To improve clinical laboratory techniques in the laboratory confirmation of respiratory viral disease diagnosis.

Methods Employed:

Results of clinical observations and procedures are correlated with bacterial, mycologic and viral isolation employing both animals and tissue culture techniques.

Major Findings:

1. The Laboratory of Clinical Investigation has confirmed, by isolation of the virus, the diagnosis of respiratory viral disease in a total of 145 in and out patients and a few selected patients from survey groups during the first 11 months of 1956. From these patients isolation of virus was accompanied in a total of 189 specimens. A grand total of 1800 specimens were examined for viruses in the laboratory.

2. The routine procedures and the experiences of this laboratory have been summarized in a report and accepted for publication.

3. A new, more rapid, and improved laboratory test for the presence of mumps virus has been developed. Using this technique, mumps virus has been, for the first time, in and repeatedly, isolated from cerebrospinal fluid and from urine in tissue culture. A total of 17 patients have been so studied and isolation made from specimens collected during the first 5 days of illness in 73%.

4. A virus, not identified as belonging to other known groups, has been isolated from 8 children ill with a common cold-like disease.

5. Virus has been isolated from 2 patients with clinically well-studied primary atypical pneumonia.

6. Extensions of the clinical manifestations of herpes virus infection have been made to include primary vesicular lesions of the face by the description of a case confirmed by isolation of the virus from vesicular fluid.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Significance to Microbiological Research:

Illustrates progress in study of respiratory disease, acute and chronic, and oro-pharyngeal ulcerative disease which are prime unsolved problems in the infectious disease field. The major advances in this field are being made in settings such as this where the efforts of research laboratories in bacteriology, mycology and virology can be correlated with clinical material under ideal study conditions.

A major effort is exerted toward clinical studies in relation to newer viruses uncovered by tissue culture techniques.

Proposed Course of Projects:

Studies will continue as outlined under "Objectives" in patients referred to The Clinical Center and in other hospital or community surveys when indicated. This project should be expanded because of the great importance of careful clinical studies on patients with respiratory illnesses in order to link these illness with viral agents that are now being isolated in a number of laboratories.

Form No. ORF-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-8(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$44,744	91,425	136,169	2.7	2.5	5.2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	5	4	9	872

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID - 8(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Utz, John P., Parrott, Robert H., Kasel, Julius A.: A Diagnostic Virus Laboratory for a Clinical Service. Accepted for publication by the Journal of the A. M. A.

(2) Cramblett, Henry G., McCullough, Norman B., Utz, John P., Burdick, William F., Parrott, Robert H.: Herpes Simplex. A Case Report of a Primary Facial Lesion with a Discussion of Laboratory Diagnosis. Accepted for publication in the Clinical Proceedings of Children's Hospital.

(3) Parrott, Robert H., Cramblett, Henry G.: Non-Bacterial Infections Affecting the Nasopharynx. Accepted for publication in Pediatric Clinics of North America, February, 1957.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 9 (c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Brucellosis
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. Grace A. Beal
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION

Objectives:

The objects of this project are broad and studies are conducted on all aspects of brucellosis, including the nutrition, metabolism, and enzymatic make-up of the organism, mechanism of action of antibiotics on the organism, factors determining virulence of the organisms, the development and efficacy of prophylactic immunizing agents, field studies to determine the occupational hazard of the disease, epidemiology, diagnosis and therapy of the disease in man.

Clinical studies concern diagnostic criteria for the disease in man, especially in borderline cases of chronic illness in which present cultural and serological techniques fail to provide reliable evidence of infection; studies of the pathogenesis of the disease; and the evaluation of current therapeutic regimens.

Methods Employed:

The Warburg Technique is employed in enzymatic and metabolic studies; growth in a synthetic medium is used for nutrition experiments; guinea pig inoculation for virulence and vaccine studies; occupationally exposed groups for epidemiological studies; human patients for studies on diagnosis and therapy.

Major Findings:

Clinical studies are hampered by lack of patient material. Additional evidence is accumulating on the reliability of current diagnostic methods. Treated patients continue to do well without relapse.

Additional evidence has been obtained regarding the correlation of culture characteristic with virulence.

The collaborative study with the Veterinary Department of Purdue University continues. Brucella melitensis remains characteristic for the species after residence in the hog. Preliminary results indicate that the hog is moderately resistant to infection with their species.

Significance to Microbiological Research:

From a public health standpoint, in the United States, brucellosis is the most important disease of animals transmissible to man, is the cause of serious economic loss to the livestock industry, is an occupational disease, and is one which presents many problems in the diagnosis and therapy of the infection in man. The organism readily lends itself to basic studies outlined elsewhere in this report.

Proposed Course of Project:

Continue studies under way.

Form No. ORI-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-9(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$39,785	44,355	84,140	1.0	4.0	5.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	4	5	426

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSIST-ANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID - 9 (c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

part A. Project Description Sheet

1. NIAID - 10(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Granulomatous Disease
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. John P. Utz, Dr. Richard K. Merchant, Dr. Donald B. Louria, Dr. Ned Feder
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases.

10. PROJECT DESCRIPTION:

A. Sarcoidosis

Objectives:

(1) Long-term clinical study of the course of sarcoidosis and the effect of hydrocortisone (and related steroid hormones) in altering the manifestations and course of the disease.
(2) Clinical studies attempting to demonstrate agents known to produce granulomatous disease in patients with sarcoidosis.
(3) Evaluation of the Kveim skin test as a diagnostic tool in the management of sarcoidosis.

(4) Study of the calcium metabolic abnormality and the effect of therapy in patients with this manifestation of sarcoidosis.
(5) Study of immunological mechanisms in sarcoidosis.

Methods Employed:

(1) Hospitalization and outpatient observation of patients with sarcoidosis.

(2) Extensive cultural and serological study of patients with sarcoid to demonstrate etiological agents.

(3) Use of Kveim antigen and a control "antigen" preparation in patients with sarcoidosis and granulomatous diseases of known etiology.

(4) Calcium balance studies on patients with sarcoidosis and hypercalcemia before, during, and after treatment.

(5) Tissue culture of lymph nodes removed from patients with sarcoidosis in an effort to:

a) study the characteristic aspects of growth of such tissue.
b) study cytotoxic effects of tuberculin and other antigens on such tissue.

Major Findings:

Approximately 25 cases of sarcoidosis have been studied here employing the above mentioned methods where applicable. Preliminary results in attempts to demonstrate known etiological agents in patients with the clinical syndrome of sarcoidosis have been successful in a few instances; however, in a majority of patients negative results have been obtained. Calcium balance studies in progress tend to show metabolic abnormalities akin to Vitamin D intoxication in patients with hypercalcemia due to sarcoid without predictable effect of hydrocortisone on these abnormalities. Early results in evaluating the Kveim test indicate that it is specific and reasonable sensitive in the acute stage of the disease. Tissue culture studies with granulomatous lymph nodes from patients with sarcoidosis and granulomatous diseases of known etiology have shown indistinguishable growth characteristics. The cytotoxic effects of tuberculin (on other antigens) on these nodes was too variable to permit generalizations.

Significance:

Sarcoidosis is a disease of unknown etiology which closely resembles granulomatous diseases of known etiology. Investigation of sarcoidosis may provide better understanding of its nature and cause and may shed light on basic immunological phenomena of tissue allergy. Study of metabolic processes, such as calcium abnormalities, in sarcoidosis provides a basis for more precise characterization of these changes and a rational guide for experimental therapy.

Proposed Course of Project:

Continuation as outlined above.

B. Systemic Fungal Diseases

Objectives:

(1) To study clinical diagnosis, pathogenesis and natural course of systemic fungus infections.
(2) To study the problem of fungus disease due to overgrowth during antibiotic and steroid treatment.
(3) To evaluate new agents in antibiotic and chemotherapeutic treatment of systemic fungus infections.
(4) To set up means of measuring blood levels of new antifungal agents and to study acute and long term toxic effects of the drugs.
(5) To study various immunologic aspects of this group of diseases.

Methods Employed:

Patients with suspected fungal infections are studied diagnostically using the skin test, serologic, histologic, and cultural methods. Family contacts are similarly investigated and environmental studies undertaken, when indicated. Infecting fungi from such patients are screened for sensitivity against a considerable number of chemotherapeutic or antibiotic agents and therapy is undertaken after such studies and where clinically advisable.

Drugs are screened for activity against an organism isolated from a patient by in vitro methods. Promising drugs are then tested in animals for acute and long term toxicity and for therapeutic effectiveness.

New methods are being developed for determining serum drug levels, rates of excretion of drug, and in vitro screening of drugs.

Major Findings:

A number of patients with a variety of culturally proved systemic fungal diseases have been studied. These include cases of cryptococcosis(7), nocardiosis(2), coccidiodomycosis(2), aspergillosis(2), blastomycosis(1), and histoplasmosis(4). Comprehensive clinical studies have been carried out to demonstrate and measure such facets of infection as adrenal, liver, and pancreatic, and brain involvement. For example, underspread infection of cryptococcus has been demonstrated where only local involvement has been previously thought to be present.

These patients have been treated with a variety of new chemotherapeutic agents and the response of infection to these drugs has been measured. In addition pharmocologic studies have yielded information about blood, cerebrospinal fluid, tissue drug levels. A new drug, endomycin (Upjohn) has been given a trial in a variety of systemic fungal infections and its usefulness has been assessed in a total of five patients. Another new drug, Amphotericin B (Squibb) has been under study and on the basis to a great extent of these studies newer, improved preparations have been prepared and are to be evaluated. These drug studies in this variety of patients constitutes a research project unparalleled in this country.

All of the above patients, who have died, have been extensively studied in the pathologic anatomy laboratories of NIH and these studies as well, constitute invaluable data. They have proved, for example, the occurrence of subacute endocarditis due to cryptococcus which fungus has never previously been reported as a cause of this disease. An additional three cases of systemic fungal infection endocarditic lesions, not clinically apparent prior to death, have been found. This finding of endocarditic lesions in these patients promises to influence conceptions of pathologic involvement by these fungi.

A serologic routine has been established for the performance of complement-fixation tests at the NIH which will enable us and other Institutes (NINDB) to evaluate the usefulness of these tests in proved and suspicious cases of fungal disease. A total of 385 specimens have been tested during the first 11 months of 1956 in this laboratory.

In the laboratory concurrent studies have been going on evaluating in animals the above and other drugs. It has been shown, for example, that sulfadiazine definitely prolongs the life of mice infected with histoplasma, thus resolving conflicting data in the literature. Studies on Amphotericin B (Squibb) have shown that this drug eradicated otherwise overwhelming infection in nearly 100% of mice infected by histoplasma and in 50% of mice infected by cryptococcus. No other drug has ever been demonstrated in vivo to be this effective against either fungus.

Tissue culture techniques have been adapted to the diagnosis of fungus infection. In one case of cryptococcal meningitis the diagnosis has been culturally confirmed by this means alone. It appears on the basis of this early experience that this will be a valuable diagnostic technique in fungal diseases.

Significance to Microbiological Research:

The group of fungus diseases under study is poorly understood; there is no uniformly successful treatment. Apparently, certain of them, such as histoplasmosis, are being recognized as quite prevalent; others such as moniliasis create problems by the very ubiquity of the organism, which may grow more abundantly and cause systemic disease in the presence of most antibiotics and steroids, creating serious and extensive clinical problems.

Proposed Course of Project:

To proceed as outlined under "Objectives" and "Methods."

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-10(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$27,521	337,640	365,161	3.3	-	3.3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	-	2	3213

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID - 10(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Louria, Donald B., Feder, Ned, Emmons, Chester W.; Amphotericin B in Experimental Histoplasmosis and Cryptococcosis. Accepted for publication in Antibiotics Annual 1956-57.

(2) Louria, Donald B., Feder, Ned: Sulfadiazine in Experimental Histoplasmosis. Accepted for publication in Antibiotics and Chemotherapy.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 11(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Hepatitis and Mononucleosis
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. Dr. Irving Kushner and Dr. Henry G. Cramblett
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH ONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION

A. Infectious Hepatitis

- Objectives:

(1) Better to define the diagnosis, differential diagnosis and natural course of infectious hepatitis and homologous serum hepatitis.

(2) To attempt to isolate and propagate the causative agents in tissue culture.

(3) To investigate factors which predispose to chronic hepatitis or post hepatic cirrhosis.

(4) To study antibody production during acute and convalescent stages of disease.

Methods Employed:

Clinical observation and clinical laboratory data are compiled on Clinical Center patients. Family contacts are examined and specimens obtained for clinical laboratory procedures in an attempt to define subicteric infection. Similar liver function tests are obtained on most of the febrile patients admitted to the Service for control and for evaluation of hepatitis in other entities. Tissue culture and virus purification techniques are employed in an attempt to isolate viral agents. Appropriate procedures are employed to assess the role of other organisms such as leptospira and bacteria in hepatitis.

Major Findings:

Attempts are being made to propagate infectious hepatitis virus in Detroit 6, bone marrow, tissue culture cells.

A few patients have been hospitalized and are being followed in long term studies to determine the value of such treatment measures as bed rest. Newer diagnostic tests such as serum glutamine-oxalacetic transaminase determination are similarily being evaluated.

Studies have been done testing acute phase serum from infectious hepatitis patients in chick embryos. This serum did not provoke the appearance of histiocytes in the chronic allantoic fluid in numbers significantly greater than those found after inoculation of control serum. This fails to confirm a previous study reporting a significant rise as a criterion of presence of virus in serums.

Significance to Microbiological Research:

The clinical spectrum of infectious hepatitis is not well defined by objective methods, but apparent infection is responsible for serious illness and death in all age groups. The frequency and clinical nature of subicteric infection is not known, but on the basis of high gamma globulin protective ability, a majority of adults have been exposed to the causative agent.

Proposed Course of Project:

To continue as outlined under "Objectives" and "Methods" on larger numbers of patients.

B. Infectio

Objectiv

To assess the degree of involvement especially that in the liver of the human patient with infectious mononucleosis; to attempt virus isolation from patients during various periods of their illnesses using fresh human tissue culture techniques; to determine metabolic and immunologic changes in patients with severe generalized viral disease; investigate for other than viral causes of this syndrome; Listerellosis, mosis.

Methods Employed:

Hospitalization of patients for study by clinical observation and laboratory methods. Laboratory methods involve primarily the newer tissue culture techniques, such as attempts to unmask viruses by the Huebner-Rowe technique.

Major Findings:

None. Too few patients are being seen to accumulate data.

Significance to Microbiological Research:

Infectious mononucleosis is a common and perhaps even universal infection of man. Certain characteristics of the disease suggest that it may be an intermediate link between infections and neoplastic blood dyscrasias. Since heterophile titers develop, the disease lends itself to basic studies in immunology.

Proposed Course of Project:

This study is temperarily inactivated due to lack of patient material. If patients become available the project will be reactivated.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-11(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$8,719	57,027	65,746	1.1	0	1.1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	0	1	546

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID - 11(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 12(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Aseptic Meningitis
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. Dr. Henry G. Cramblett and Dr. Thomas F. Dolan
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases.

10. PROJECT DESCRIPTION:

Objectives:

a) To develop methods and use newly available methods to diagnose the large group of non-bacterial meningo-encephalitides.

b) To correlate etiological findings with detailed historical, physical and clinical laboratory observations.

c) To further define existing etiological entities and to discover new etiological entities in this group of diseases.

Methods Employed:

Hospitalization of patients for clinical observation and laboratory studies. Laboratory procedures include: a) tissue culture and suckling mouse inoculation of material for virus isolation; b) appropriate serological procedures with acute and convalescent serum for the known viral meningo-encephalitides, Leptospira, Toxoplasma and infectious mononucleosis. A number of patients selected from other hospitals or surveyed groups will be studied similarily.

Major Findings:

1. The diagnosis of non-bacterial meningitis was confirmed by the isolation of virus from 19 of 20 patients hospitalized. This high percentage of isolations reflects examination of many specimens and careful handling and prompt delivery to the laboratory of these specimens.

2. The etiology relationship of Coxsackie A - 9 virus to Aseptic Meningitis has been established by the isolation of this agent from cerebrospinal fluid of a patient with this syndrome.

3. A variety of additional viruses previously reported as rarely isolated from the cerebrospinal fluid have been repeatedly isolated from this source under optimal conditions described above.

4. Patients infected by one group of these viruses (Coxsackie) have been intensely studied to characterize the spectrum of clinical illness.

Significance to Microbiological Research:

Non-bacterial meningitis is a serious disease of whose etiology and pathogenesis there is little knowledge. It often results in brain damage and behavior disturbances. Increased knowledge may also clarify the pathogenesis of the encephalitic complications of the otherwise minor diseases of children.

Proposed Course of Project:

Continue above studies.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-12(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$23,265	26,251	49,516	2.0	2.5	4.5

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	-	1	246

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID - 12(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 13 (c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Allergy-Immunology
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Drs. Harry G. Steinman, John P. Utz, and James L. German
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLIMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in The Laboratory of Infectious Diseases, NIAID, The Laboratory of Immunology, NIAID, and The Laboratory of Biochemistry, NCI.

10. PROJECT DESCRIPTION:

Objectives:

1. To evaluate the role of immune serum globulins in host response to infectious diseases.

2. To evaluate the role of bacterial, fungal, or viral allergens in recurrent or chronic respiratory disease.

3. To determine the rate of metabolism of individual antibodies compared to that of gamma globulin, and to investigate the mechanism whereby the reticulo-endothelial system differentiates between otherwise chemically homogeneous specific antibodies.

4. To measure, quantitatively, the rate of synthesis of specific antibody in the human during the primary and secondary immune responses.

5. To measure quantitative differences in the specific antibody response of acute leukemics, normal human volunteers, hypo-and hypergammaglobulinemias.

Methods Employed:

Clinical studies supplemented with:

1. Plasma protein analysis by (a) electrophoresis both by paper chromatography and moving boundary technique, (b) chemical determinations, and (c) immunologic assays.

2. Immunization and testing of response by serologic techniques or by challenge.

3. Immunochemical procedures by the Kabat and Mayer methods of analysis of specific precipitates and by the Oudin diffusion technique.

4. Isolation and investigation of individual gamma globulin antibodies by ion-exchange fractionation.

Major Findings:

One of the most important findings listed in the 1955 Report was the observation that, in hypogammaglobulinemics, pooled passively-infused antibodies are metabolized at very different rates in the same individual at the same time but that each individual antibody is metabolized at a characteristic rate.

Tolerated below are the rates of metabolism of five different antibodies occurring in one batch of pooled gamma globulin:

Antibody	Half-life (days)
Total gamma globulin	36
Poliomyelitis (Type I) (Neutralizing)	
Poliomyelitis (Type II) (Neutralizing)	58 ± 15
APC Type II (Neutralizing)	43 ± 1.5
Tetanus (Antitoxin)	41 ± 3
Diphtheria (Antitoxin)	28 ± 5
Coxsackie A (Neutralizing)	17.5 ± 2

By means of ion-exchange chromatography it has been possible to separate crude gamma globulin into a number of fractions with different physico-chemical properties and which are enriched in specific antibodies. This technique gives promise of yielding purified individual antibodies - material heretofore unavailable.

Significance to Immunologic Research:

This project exemplifies the use of clinical material in basic immunologic research. Knowledge resulting from such studies may be expected to increase our understanding of the infectious and allergenic diseases and ultimately lead to improved clinical management of patients with such diseases.

Proposed Course of Project:

These studies will be continued and extended. Specific antibodies in normal patient convalescent sera will be investigated to complement the studies on passively immunized hypogammaglobulinemics.

The mechanism of delayed allergy (tissue immunity) in hypogammaglobulinemic patients will be studied.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-13(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$6,894	22,630	29,524	0.6	-	0.6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	-	1	220

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NI ID - 13 (c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Martin, C. M., Gordon, R. S., and McCullough, N. B. : Acquired hypogammaglobulinemia in an adult. Report of a case, with clinical and experimental studies. New Eng. J. Med., 254:449-456 March 8, 1956.

(2) Martin, C. M., Gordon, R. S., Folts, W. R., and McCullough, N. B: Studies on Gamma Globulin - I. Distribution and Metabolism of Antibodies and Gamma Globulin in Hypogammaglobulinemic Patients. J. Clin. Med. (In press)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 14 (c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA

6. Enteric Diseases
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. Thomas L. Gorsuch and Miss Rose Lieberman
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION:

Objectives:

Basic studies of the etiology, pathogenesis, and immunology of enteric diseases which include:

1. Fundamental laboratory investigations of the immunogenic abnormalities of the Salmonella.

2. Clinical study of the mechanisms and effects of enteric infections on the host which lead to the "carrier state".

3. The evaluation of antibiotic and surgical therapy on the "carrier state" of Salmonellosis.

Methods Employed:

Animal protection studies using passive immunization techniques to determine the immunological differences in the vaccinated individual, in the "carrier state", in recovery after active disease, and in the newborn child before exposure to exogeneous antigen sources.

The fractionation of the components of the antibodies by absorption and adsorption using the Oudin techniques, hemagglutination tests, quantitative precipitin procedures, chromatography, and electrophoresis.

Experimental therapy of the typhoid carrier state and other salmonellosis in man.

Major Findings:

Basic studies: Changes in the protective titer of the antibodies that may be brought about by the spatial disarrangement or alteration of the original configuration of antibodies, are presently under detailed study. Evidence has been obtained to show that certain components of Salmonella antibodies may enhance, inhibit, or have no effect on the protective mechanism.

Clinical studies: A number of patients with acute and chronic enteritis were studied to determine what pattern of immunity existed in these people in addition to the etiology of the infections.

Study of the therapeutic value of a variety of combinations of antibiotics continued. A small number of patients have been apparently cleared of its carrier state.

Significance to Microbiological Research:

Outbreaks of enteritis, are of major importance, particularly when children are involved. Carriers who have chronic asymptomatic infections are usually responsible for these outbreaks, and are a serious public health problem. The carrier state does not yield to present methods of therapy.

Proposed Course of Project:

Etiologic studies on patients from epidemics and sporadic cases of enteritis will continue.

Major emphasis will be placed on an investigation of the etiology and treatment of the carrier state in Salmonella and Shigella infections. Extensive therapeutic investigations are planned utilizing antibiotics, singly and in combination, and measures which will alter the immunologic state of the host.

Basic studies: The results of the initial investigations of the mechanism of immunity to Salmonella infections are presently being prepared for publication. Several other facets of this same problem as related to the carrier state, to immunity following TAB vaccination, to passively immunized new-born infants, to patients who have recovered from active infection, are being studied

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-14(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$30,865	77,847	108,712	2.0	3.0	5.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	3	4	740

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID - 14 (c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 15(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Helminthic Infestations
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe, Miss Elizabeth Guinn
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

(1) Some part of the studies on human filariasis is done in collaboration with and complements investigations carried out by the Sections of Medical Entomology and Helminthic Diseases, Laboratory of Tropical Diseases. Cooperation also obtains with the Student Health Service, Howard University.

(2) In the Intestinal Nematode Infestations, there is close cooperation with the Section of Helminthic Diseases, and Section of Immunology, Laboratory of Tropical Diseases, NIAID. Patient material is obtained through a cooperative arrangement with the Medical Branch, Department of State.

(3) The studies dealing with Trematode Infestations supplements projects on Trematodes, especially Schistosoma, being carried out by the Laboratory of Tropical Diseases. These are Chemotherapy of Parasitic Diseases, Foreign Field Stations, Fundamental Physicological and Biochemical Studies on Parasites, and Studies on Helminths and Diseases Caused by Them.

(4) The Infestations due to Cestodes supplements and is correlated with investigations being carried out by the Laboratory of Tropical Diseases.

10. PROJECT DESCRIPTION:

A. Filariasis

Studies concerning human filariasis, especially infections with Wuchereria bancrofti.

Objectives:

1. To clarify factors involved in host reaction and the existence of hypersensitivity in persons infected with W. bancrofti.

2. To elucidate the mechanism of action of "Hetrazan" on microfilariae and possible effects on adult worms.

3. To determine precipitating factors in the production of attacks of filarial lymphangitis.

4. To explain the mechanism of periodicity of microfilariae.

5. To determine if transmission of the disease is occurring in the United States, especially the Washington area.

6. To develop procedures and methods whereby basic studies can be done more effectively.

7. To study pathogenesis and experimental therapeutics of elephantiasis.

Methods Employed:

Through a cooperative arrangement with the Student Health Service, Howard University, night specimens of blood are collected from each new student being admitted to Howard University who previously resided outside the United States. Those found to be positive for microfilariae or who have a suggestive history of filariasis, are considered as

potential candidates to the project. Through the year, they are then admitted to the Clinical Center for observation and study. Basic work up consists of the routine study for admission, one or two days and night study of microfilariae periodicity, x-rays of soft tissue, especially the femoral area. Additional special studies then are carried out depending upon the results and where the candidate would best fit into a protocol of special studies devised to meet the objectives as listed.

Major Findings:

1. The mere fact that filariasis due to W. bancrofti is present in from two to ten per cent of foreign students coming mainly from endemic areas for W. bancrofti and that Culex quinquefasciatus, the main vector, is prevalent in the Washington area is a finding of major importance.

2. Microfilariae will remain viable for periods of at least a month when blood is collected in heparinized or oxalated tubes and stored at four degrees centigrade.

3. Infective filarial larvae stages developed in Culex quinquefasciatus, when the mosquitoes were fed on a carrier of a very low density of infestation (estimated 0.7 microfilariae per 20 cubic millimeters.)

4. Despite present beliefs to the contrary, hetrazan may act directly on microfilariae. There is also a suggestion that the drug may also aid in an agglutination phenomenon of microfilariae.

5. More detailed laboratory and clinical information has been collected to describe the immediate reaction following the administration of Hetrazan.

SIGNIFICANCE TO MICROBIOLOGICAL RESEARCH

The program is of considerable interest to the Institute for a number of reasons: The fact that carriers are in the United States, as well as suitable mosquito vectors which could conceivably help the infection become established within the U. S.; an estimated 186 million people in the world are infected, and the United States is committed to the expenditure of rather large sums of money in other areas in programs aimed toward the eventual control of the disease. The results described above, particularly the prolongation of viability of microfilariae in low temperatures, open up many possibilities for more adequate study of this disease, and perhaps for fundamental host reaction studies associated with hypersensitivity. Leads on the mechanism of action of hetrazan are of great fundamental importance.

Proposed Course of the Project:

Arrangement with the Student Health Service, Howard University, will continue through next year. Staff and personnel permitting, we would like to survey migratory farm workers being imported from Trinidad. Preliminary discussion has been made with the Department of Labor, and we have secured their cooperation whenever we wish to move into this area.

The possibility exists of one of the investigators spending three months in India as a consultant to the Filariasis program being carried out in that country. (Fiscal year 1956 United States commitment 1.5 million dollars, and Indian government dollar equivalent 3.5 million to go to 20.0 million in fiscal year 1957). If this materializes, microfilaria, adult worms, and other material will be sent to the National Institutes of Health for additional studies.

Because of the smallness of our staff, and the number of projects under our responsibility, the bulk of our efforts has been in establishing perimeters in this area of parasitic and tropical diseases. Most certainly, insofar as possible, attempts will be made to follow up the leads as indicated.

B. Intestinal Nematode Infestations

Objectives:

To correlate clinical manifestations and pathology during the various phases of the life cycle of nematodes in the mammalian host, especially man; to describe their role in the production of disease; to evaluate the efficacy of established or newer antihelminthics; to investigate differences in species in terms of their clinical attributes; to improve diagnostic techniques, and to elucidate the extent and severity of infestation in the United States.

Among intestinal nematode infestations, the program embraces those species whose life cycles include a period of tissue invasion and pulmonary migrations (Ascaris lumbricoides, Strongyloides stercoralis, and hookworm) as well as those species which do not exhibit a pulmonary cycle (Enterobius vermicularis and Trichuris trichiura). Reactions of the "tissue larva migrans" type are also of interest.

METHODS EMPLOYED:

Parasitic screening of State Department employees returning to the United States and of patients referred by private physicians or governmental organizations, routine stool examinations on inpatients

under study in LCI, laboratory diagnostic techniques, immunological studies, and therapeutics. In addition an epidemiologic study was undertaken of the factors associated with continued reinfestation of a highly infested family.

Major Findings:

1. Epidemiologic data showed there to be an association between a highly infested family with multiple intestinal parasitism, and many episodes of pneumonitis among the children, and the use of sewage sludge as fertilizer. Eggs of human parasites were found in the family yards, in sewage sludge ready for distribution as fertilizer from two sewage treatment plants in the Washington area, and on a university campus where sludge was being distributed on the lawn.

2. Therapeutic trial and follow-up studies were carried out on a few patients with ascariasis, enterobiasis, strongyloidiasis, hookworm, and trichostrongylus. Three patients with the latter parasite were observed.

3. Three cases of Trichostrongylus s.p.p. were observed among State Department employees returning from the Middle East.

SIGNIFICANCE TO MICROBIOLOGICAL RESEARCH

The demonstration that the present practice of disposing of sewage sludge, may be a potential serious public health hazard brings some degree of prestige to the Institute, and emphasizes that the time is not yet ready for complacency in consideration of any communicable disease. The actual health hazard resulting from this practice, except under the special circumstances surrounding the family investigated, is not yet clarified. The fact that these practices are going on in the Washington area provides an opportunity for epidemiologic investigation of great importance to those mainly interested in environmental sanitation.

The intestinal nematodes, especially ascaris and enterobius, are common in the United States. No adequate therapy has yet been devised for the latter, and continued therapeutic investigation is certainly of significance.

Proposed Course of the Project:

Because of the smallness of our staff, we shall probably do no further epidemiologic studies developing from these leads. However, preferably through a cooperative arrangement with the local health department, we may treat all the members of the infested family and try to work out some methods of preventing reinfestation. Work will continue in a small way on therapy of the other intestinal nematodes. Basic material will continue to be collected on Trichostrongylus.

C. Trematode Infestations

Objectives:

1. Schistosomiasis.
2. Other fluke diseases.

Besides schistosomiasis, this project includes paragonimiasis and other fluke diseases.

To elucidate systemic reactions to the infestation, immunologic responses, and pathologic reactions in the organ or systems particularly affected; to evaluate new or experimental prophylactic or therapeutic drugs; to improve diagnostic techniques.

Methods Employed:

Field surveys of selected groups of people for trematode infestations, parasitic screening and work-up of patients referred by private physicians or governmental organizations for suspected trematode infestation, parasitological methods of diagnosis applied to feces, urine, bile, serologic studies, including appropriate skin tests, and reactions between ova and patient serum; clinical studies on proved cases; experimental therapy.

Major Findings:

Major emphasis has been the study of the use of Miricil D (Hilcidan) in small dosages for a long period in infected patients. The number of patients is still too small to warrant conclusions.

SIGNIFICANCE TO MICROBIOLOGICAL RESEARCH

The demonstration of an effective oral prophylactic for Americans or other persons going into an endemic area would be a great contribution. Miracil D, or an oral preparation which concentrates in the skin offers possibilities along this line. Investigations during the past year had two immediate objectives in mind: (1)evaluate the toxicity of the drug when taken in small doses for a long period of time, and (2) study of its effectiveness in clearing infestation in these dose ranges.

The great migration of infected Puerto Ricans into the United States, the importance of schistosomiasis as a major disease in most parts of the world, the fact that its prevalence continues to increase, the realization that the United States is spending many millions of dollars in endemic areas, and the knowledge that most aspects of the disease are still unknown, emphasizes the significance of this program.

Proposed Course of Project:

As time and staffing allows, to instigate surveys among groups of people coming to the United States from endemic areas. Depending upon the above to emphasize the search for a suitable prophylactic.

D. Infestations Due to Cestodes

Objectives:

To determine the extent of infestations with cestodes among specific population groups, such as the American Indian, by laboratory and immunological tests; to study clinically selected or referred patients; to evaluate therapeutic regimens.

Taenia saginata (beef tape worm) and Taenia solium (pork tape worm) are of principal interest. In infestations due to adult worms, studies are centered chiefly around the altered physiology of the bowel and therapeutic regimes directed toward eradication of the worms.

In disease produced by larvae, the clinical investigative patterns will be influenced greatly by the organs or systems chiefly involved.

Other species of cestodes are of general interest, such as H. diminuta, H. nana and E. granulosa.

Methods employed:

Field surveys of selected groups of people as indicated, parasitic screening and workup of patients referred by private physicians or governmental organizations for suspected cestode infestations, laboratory study, immunologic techniques, and experimental therapy. During the past year, observations have been started on the use of the antihistamines as agents against the tapeworms.

Major Findings:

No definitive major findings to report because of lack of sufficient patient material.

SIGNIFICANCE TO MICROBIOLOGICAL RESEARCH

Infestations with T. saginata and T. solium are difficult therapeutic problems, and are not uncommon in the United States. Information on treatment regimens would be welcomed by many practicing physicians. Cases of Echinococcus granulosa, especially among Eskimos and Indians, present diagnostic and therapeutic problems which, because of the increasing work in tuberculosis among these people, are assuming much greater importance than previously.

Proposed Course of Project:

If time, staff, and relations can be developed to initiate a parasitic survey among Indian population, a larger clinical program may be developed.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-15(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,959	5,431	17,390	0.7	1.2	1.9

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	-	1	48

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID - 15(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Beye, Henry K., Kayhoe, Donald E., Smith, Richard, and Guinn, Elizabeth: Observations Concerning A Family With Multiple Intestinal Parasitism Possibly Associated With Use Of Processed Sewage Sludge As Fertilizer. Presentation to the Sub Committee On Waste Disposal, National Research Council, September 17, 1956.

(2) Beye, Henry K., Mille, R., Thooris, C., and Tapu, John: Preliminary Report on the Use of the Antigen of Dirofilaria Immitis as an Epidemiologic Tool and as a Therapeutic Agent in Wuchereria Bancrofti Infections in French Oceania. Published in The American Journal of Hygiene, Vol. 64, No. 1, 23-29, July, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID -16(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Protozoal Infections
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe and Miss Elizabeth Guinn
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

(1) The therapeutic aspects of the amebiasis complements investigations described in project, "Epidemiology of Parasitic Diseases", Laboratory of Tropical Diseases, in that one of objectives in each project is the evaluation of various experimental therapeutic regimens. The two projects, however, are very difficult in the methods employed and in the populations studied.

Cooperation is close between this project and Laboratory of Tropical Diseases projects on Chemotherapy of Parasitic Diseases, Nature of Immune Responses in Parasitic Diseases, and Studies on Amoebiasis. There is, likewise, close cooperation with the Medical Branch, Department of State.

(2) The project on trichmoniasis complements, and is a part of the trichmoniasis program, being carried by the Laboratory of Tropical Diseases. It concerns itself specifically with the parts of that project involving patient responsibility.

(3) The project on toxoplasmosis complements and supplements the following projects in the Laboratory of Tropical Diseases: "The Nature of the Immune Response in Parasitic Diseases", sub-title, "Studies on the Role of the 'Accessory Factor' in the performance of the Dye Test for Toxoplasmosis"; Studies on the Biology and Pathogenesis of Protozoan Parasites of Man, sub-title, "Studies on Toxoplasmosis"; Epidemiology of Parasitic Diseases, sub-title, "Epidemiology of Toxoplasmosis", and sub-title, "Biological Studies of Toxoplasma gondii". There is, in additi close cooperation with the National Institute for Neurological Diseases and Blindness.

(4) The project on Blood and Protozoan Diseases, exclusive of toxoplasmosis, works in close cooperation with appropriate projects in the Laboratory of Tropical Diseases, notably, Chemotherapy of Parasitic Diseases

10.. PROJECT DESCRIPTION:

A. Amebiasis

Objectives:

To improve and standardize diagnostic techniques for infections due to Endamoeba histolytica; to determine adequate criteria for the diagnosis of amebiasis; to investigate the difference in strains or ra of the parasite in correlation with clinical attributes; to study the role of associated bacterial flora; and to study of clinical and path- ological manifestations of the disease in man and its pathogenesis; and to evaluate various experimental therapeutic regimens including the establishment of valid criteria of cure.

Methods Employed:

Field surveys of suitable groups of people for amebiasis, parasitic disease screening and work-up of patients referred by private physicia or governmental organisms as suspected cases of amebiasis, clinical study of proved cases, laboratory diagnostic techniques, immunological studies, experimental therapy.

Since March of 1956, major emphasis in procuring patient material for this project has been through a cooperative arrangement with the Medical Branch, Department of State, whereby our laboratory screens stool specimens of each State Department employee returning to the United States. Under this arrangement, each employee fills in an epidemiologic questionaire when his first specimen is submitted. Those found to be positive for E. histolytica, and a suitable candidate for study, are then submitted to the project for further work-up and specialized studies as the case may be. At the inception of the cooperative program, attempts were made to collect from three to five specimens from each employee, before ruling out infection, but because of the fact that increased technical help, expected at

the inception of the program did not materialize during the year, in May we changed the number of specimens to only one per person. Even under this procedure, more than fifty candidates have come from this source alone.

In general, such candidates are handled in the Admission and Follow-Up Department, where they routinely receive a history and physical, Chest X-ray, routine hematological study, liver functions, complement fixation tests for amebiasis, routine serology, and urine studies. Barium enema, X-ray, and proctosigmoidoscopy are done when indicated. Treatment schedules of interest this past year have been a combination of tetracycline and chloroquine diphosphate, and erythromycin sterate. Follow-up observations are attempted for a year. Candidates having special areas of interest, or needing hospitalization are admitted as inpatients. Our most interesting patient in this respect has been an extremely complicated one with amebic liver abscess.

Major Findings:

Inasmuch as time and staff will allow, a system for tabulation, and summarizing the data obtained through the use of the epidemiologic questionnaire, the various schedules for clinical and laboratory observations, and follow-up studies, are gradually being set up. This is not yet in full operation, and because of this, the following discussion on major findings will be based on a qualitative knowledge of the data.

Tetracycline and chloroquine diphosphate give a higher percentage of cures than erythromycin sterate, although even with the former drugs, infection can persist. Erythromycin, in fact, under certain conditions, yet unexplained, seems to cause an activation of the infection, with the expulsion of many trophozoites. This provides a lead, now under further study, both for a refined diagnostic tool in patients with light infections, or for a clarification of the role of bacterial associates in the pathogenesis of the disease. Another lead, now under further study, is the possible relation of erythromycin therapy and the elevation of alkaline phosphatase levels, first observed in our patient with amebic liver abscess.

Generally, the symptomatology of patients we have observed with amebic infections is minor, although present. As illustrated in the case of amebic liver abscess now under study it can be very serious and still present little laboratory or clinical evidence of the severity of the condition. In this case, it is believed that correlations will be found between amebiasis complement fixation titers, clinical manifestations, and perhaps alkaline phosphatase levels.

Significance to Microbiological Research:

Without going into great detail about the importance of amebiasis, as a potential health hazard to the people of the United States (2 to 10% of the population is infected with E. histolytica, and pathogenesis, leading to severe fulminating condition, is not understood), this project is at the present time, the backbone of patient procurement for the Parasitic Disease Service program. Our experienc of depending almost exclusively upon private physician referrals for patient material (from 1953 until 1955) was not entirely satisfactory. Lack of adequate private laboratories in the Washington area created a tendency of referrals here for diagnostic work-ups, which often proved disappointing from our point of view, but involved a more elaborate screening than has to be conducted under the present relation with the State Department. We still accept private physician referrals, but depend upon the survey. On the basis of this cooperation, we now have first choice on all other types of diseases of interest to the Institute. The amebiasis diagnostic techniques and criteria remain unsatisfactory, and therapeutics remains largely experimental. Leads as described utilizing erythromycin have significance to the general problem of amebiasis and may have special bearing on the major problems of the use of erythromycin for any infection. Detailed clinical and laboratory observations such as are being collected on the case of amebic liver abscess give new emphasis on the complement fixation test, the alkaline phosphatase test, and even the clinical pic ture of this complication of intestinal amebiasis.

Proposed Course of Project:

It is expected that within the next few months, the Medical Branch, Department of State, will employ a technician, who will receive additional training in parasitology in our laboratory, who will then carry on the parasitic screening at the office of the Medical Branch. We shall continue to receive a review of those persons found to be positive for E. histolytica for selection of those of interest to us. This move will release our small staff for work with more immediate research implications. In general this will consist of investigations to improve the present methods of diagnosis, continuation of studies already described, and if possible (staff or collaboration) to shift our emphasis to a more intensive study of selected patients with E. histolytica in investigations to clarify the respective roles of nutrition, intestinal flora, and perhaps minor intestinal trauma, in the pathology and symptomatology of the disease.

Efforts will be made to have our statistical organization (one girl part time at present) functioning so that adequate follow-up studies can be facilitated. With recent reports of retinitis associated with amebiasis therapy, efforts will be made for cooperativ studies with the opthalmology section of the National Institue of

Neurological Diseases and Blindness for study of this new aspect of the disease.

B. Trichomoniasis

The total trichomoniasis program consists of five parts: (1) prevalence surveys, (2) clinical observations on infected volunteers, (3) pathophysiology of the vaginal tract, (4) evaluation of treatment, and (5) epidemiologic studies.

Objectives:

To define the extent of trichomoniasis and moniliasis among a population of working women; to correlate, if possible, the association of trichomoniasis and abnormal vaginal cytopathology and genital cancer, to gain epidemiological data concerning the disease; to correlate signs and symptoms of infection and presence of Trichomonas vaginalis and Monilia albicans; to ascertain the relationship of laboratory and clinical findings during various phases of the menstrual cycle to infection with trichomonas and/or monilia; to evaluate various therapeutic regimens.

Methods Employed:

Clinical, laboratory, and epidemiologic studies of the N. I. H. employees found to be positive for Trichomonas vaginalis; therapeutic trials of various treatment regimens; and epidemiologic studies of family contacts.

Major Findings:

1. Ten of forty-one women, treated with one or more of the trichomonicidal drugs were parasitologically free of the infection three months after cessation of treatment.

2. In women with a committant infection with Monilia albicans, cure was obtained through treatment with one or more of three drugs.

3. Most effective treatment to date consists of a combination of vaginal and oral aureomycin, in which five of fourteen women revealed parasitologically negative cultures three months after treatment. Seven additional patients were treated with aureomycin and propion gel. In these only one became parasite free, and this was not until near the end of the aureomycin therapy.

Significance to Microbiological Research:

Trichomoniasis is a very common and occasionally severe infection among a large percentage of women in the United States. Little is known of its epidemiology, especially factors involved in transmission, and its clinical spectrum remains unclarified. Despite the results listed above, treatment remains unsatisfactory.

Proposed Course of Project:

When this cooperative project was established last year, staff commitments were made for one year, and with an understanding that increased staff was necessary because of an expansion of the prevalence survey among female employees at the Health, Education and Welfare buildings. Inasmuch as this aspect of the program has not developed, we believe a group appraisal of the program is now warranted. This is especially pertinent because our own staff has many interesting leads in other fields not being followed up at present on account of the demands of this particular project.

C. Toxoplasmosis

Objectives:

To clarify the natural ecology of the disease in humans; to correlate clinical manifestations and pathogenesis; to evaluate diagnostic methods; and to determine effective methods of therapy.

Methods Employed:

Serologic surveys; clinical study and observation; screening and parasitic study of patients referred by private physicians or governmental organizations as suspected cases of toxoplasmosis, laboratory and immunologic diagnostic investigations, and experimental therapy.

Major Findings:

A great deal of previously unknown knowledge was obtained by detailed study of two cases of toxoplasmosis proved by the isolation of the parasite from various tissues and body fluids, which incidentally comprise the largest treated series of acquired disseminated toxoplasmosis on record. The first of these was a laboratory acquired infection, while the second represented the first case in which eye manifestations were observed in the acute phase of the disease. Data such as specific incubation period, observations on clinical manifestations, parasitemia, serological responses and changes, serum protein patterns, and observations following initiation and during pyrimethamine therapy constitute a series of major findings.

Significance to Microbiological Research:

There is evidence of high prevalence judging from high precentage of positive immunologic responses. The clinical spectrum of the disease needs much clarification. It may well be associated or confused with a variety of conditions including reticulum cell sarcoma, (one of our patients was admitted to the Clinical Center as a case of lymphatic leukemia) thyroiditis, Hodgkins disease or infectious mononucleosis. It is known to cause blindness and congenital central nervous system changes. The epidemiology is still mainly unknown, and the efficacy of specific drugs need further clarification.

Proposed Course of Project:

If at all possible through increase of staff or collaborative efforts, to initiate further epidemiologic and serologic surveys, in an effort to discover additional patient material. To continue basic laboratory and clinical observations as indicated.

D. Blood and Tissue Protozoan Disease

Objectives:

To improve diagnostic techniques; to study the clinical and pathological manifestations of these diseases in man and their pathogenesis; to evaluate various experimental therapeutic and prophylactic regimens.

Methods Employed:

Field surveys of groups of people, as indicated; parasitic screening and work-up of patients referred as suspected cases (malaria, leishmaniasis, Chagas disease) by private physicians or governmental organizations; clinical study of proved cases; laboratory diagnostic techniques, including cytodiagnosis; immunologic studies; laboratory culture of the organisms; and experimental therapy.

Major Findings:

The number of cases was too small to warrant definite conclusions.

Significance to Microbiological Research:

The study of these groups of diseases has significance in terms of their prevalence, and of the application of newer fundamental concepts and techniques to studies of host-parasite relation, immunological reactions, pathogenicity and therapy.

For the next few years emphasis in malaria may well be toward worldwide eradication of the parasite. Problems may develop in respect to drug reactions and resistance under the extremely variable conditions inherent in such a large program.

The first case of Chagas disease in the United States was reported last year. Inasmuch as suitable arthropod vectors for the transmission of the disease are distributed throughout most of the southwestern and western parts of the United States, this disease may assume national importance.

Proposed Course of Project:

As time and staffing allows, to obtain information on the epidemiological and clinical aspects of the diseases, and then proceed as described.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-16(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	20,746	63,364	84,110	1.3	1.8	3.1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	3	4	603

13. BUDGET ACTIVITY:

RESEARCH [X]
REVIEW & APPROVAL [ ]
BIOLOGIC STANDARDS [ ]
ADMINISTRATION [ ]
PROFESSIONAL & TECHNICAL ASSISTANCE [ ]

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID -16(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Kayhoe, Donald E., Beye, Henry K., and Jacobs, Leon: Acquired Toxoplasmosis: Observations in two parasitologically proven cases treated with pyrimethamine and triple sulfonamides. (In preparation)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 17(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Bacteriological Studies
PROJECT TITLE

7. Dr. Lewis E. Gibson
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION:

Objectives:

In selected patients to study the bacterial and fungal flora particularly of the respiratory and intestinal tracts in relation to, (a) modification of flora by antibiotics and chemotherapeutic agents, (b) sensitivity of organisms to various antibiotics, and (c) prevention of the overgrowth of undesirable organisms and resultant side-reactions.

Methods Employed:

Patients with cystic fibrosis of the pancreas will be employed in this study. Long-term studies of the bacterial flora of the respiratory and intestinal tracts will be employed. Findings will be correlated with use of antibiotics and fungistatic agents, with antibiotic sensitivities of pathogenic microorganisms isolated, in particular the Staphylococci; persistence of strains of Staphylococci will be determined. A variety of metabolic studies are performed in an attempt to understand the increased pathogenicity of these organisms in this group of patients.

In particular the abnormality of the sweat and its production is being investigated in an attempt to determine the nature of the defect. Sweat is produced by heat or the use of drugs and its chemical content determined and the results correlated with rate of sweating and manner of induction of sweating. Measurement of the sweating pressure and the histochemistry of the sweat glands will be undertaken.

Major Findings:

From our bacteriological and fungal studies we have made the following observations: a) the strains of Staphylococci found in the respiratory tract of patients with cystic fibrosis of the pancreas, as identified by antibiotic sensitivity and phage typing, tend to remain relatively constant. b) the Staphylococci found in an individual patient are frequently not resistent to the antibiotic which the patient is receiving even though he may have received it over a period of many months in a fully adequate dosage.

In studying the sweat, we have developed a rapid screening test for cystic fibrosis of the pancreas. Unknown to us a similar test was simultaneously developed elsewhere. These two techniques are being compared.

We have measured the sweat produced by ionophorised adrenalin and have found that adrenergic sweating is more profuse and prolonged than is generally appreciated. Methods have been developed to determine the effect of ionophoretically introduced cholinergic and adrenergic stimulation and blockade on the concentration of chloride in the sweat of normals and of patients with cystic fibrosis. These data are not yet extensive enough to allow conclusions in regard to the control of chloride concentration.

Significance to Microbiological Research:

With increasing availability of antibiotics, there is great need for basic guidelines for antibiotic resistant strains of bacteria. Overgrowth of undesirable bacterial and fungal organisms with resultant side reactions and sometimes fatal complications become an ever-present menace, from which medicine must protect itself and the patient.

Fibrocystic disease of the pancreas (mucoviscoidosis, exocrinosis), is a recessively inherited disease manifest physiologically and clinically in any or all of the following ways:

(1) Increased viscidity of secretions from the exocrine organs (pancreas, mucous glands, certain salivary glands) with consequent:

a. Insufficiency or absence of enzymes from the pancreas, resulting in poor digestion of food, passage of large bulky stools and undernutrition, and

b. Thickened bronchial secretions associated in time with frequent severe respiratory infections, and debilitating chronic lung disease.

(2) Increased excretion of sodium and chloride in the sweat and saliva, which can result in severe heat prostration.

(3) A peculiar susceptibility to infection with Staphylococci.

The incidence of the disease is 1 to 1.7 cases per 1000 live births and it is estimated that one in 20 well persons carries the recessive gene. The specific cause is not known. The diagnosis can be suspected clinically in many cases but newer methods of diagnosis based on the physiological defects mentioned are uncovering many variants from the classical case. The prognosis is still poor despite the use of antibiotics in suppressing infection.

Clinical treatment is directed toward:

(1) Maintaining nutrition
(2) Prevention and suppression of infection
(3) Prevention and alleviation of chronic lung disease

Clinical research on the subject is also directed toward the accomplishment of these ends. Clinical and basic research is directed toward such problems as the basic cause and cellular nature of the defect and the factors that make the children peculiarly susceptible to staphylococcal infection.

Proposed course of Project:

To continue and extend the bacteriological studies; to determine absorptive ability of fibrocystic patients for antibiotics and selected food substances; to continue the studies on mechanism of sweating and sweat content; to undertake histochemical studies of the sweat glands; to continue to study methods of clinical management of these patients.

Form No. ORP-1
October 1956
(Attachment 1)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. MIAID-17(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,810	48,881	59,691	1.1	-	1.1

	BUDGETED POSITIONS			ESTIMATED DATA
	PROF	OTHER	TOTAL	
FY'57	2	-	2	462

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGICAL STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards & Publications 15. NIAID - 17(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

(1) Silver, Richard T., Utz, John P., Frei, Emil, Zubrod, Charles G., McCullough, Norman B.: Infections, Fever, and Host Resistance in Acute Leukemia. Presented at the International Congress of Hematology, September 1, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - BB (c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Biochemical Studies
PROJECT TITLE

7. Dr. Harry G. Steinman
PRINCIPAL INVESTIGATOR

8. Dr. Eskin Huff, Dr. Lewis E. Gibson, and Dr. Irving Kushner
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION:

Objectives:

The general program deals with the biochemical aspects of infectious disease processes at both the cellular level and the clinical patient level. The goal is to obtain information leading to rational approaches to therapeutic measures.

To study the host-parasite relationships at the cellular level, research is being directed at the parasite and at the host cell as well as the interaction of the two which leads to disease.

Biochemical studies of patient material are conducted so that the biochemical changes that accompany infectious disease processes may be used for diagnostic purposes.

Methods Employed:

A. Basic Studies:

1. Nutritional, metabolic, and enzymatic studies of mammalian cells in tissue culture and of other systems derived from whole tissues, e.g., liver, kidney, etc. These experiments are designed to provide insight into the mechanisms of the biosynthesis of carbohydrates, lipides, nucleic acids, and proteins.

2. Nature of viral infection. The effects of the presence of a parasitic virus on the metabolism of a host cell are being investigated using mammalian cells adapted for growth in tissue culture.

3. Nature of viral multiplication. Techniques are being evolved for the isolation and purification of viruses and accompying by-products, e.g., "soluble" antigens, grown on tissue cultures of mammalian cells. Kinetic studies of viral growth are being made. An APC virus grown on monkey kidney host cell is one model system. Another model system is E. coli bacteriophage on its specific host bacterial cell.

4. Chemotherapy of viral infection. As a corollary of the nutritional and metabolic studies of the parasite and host cell systems described above, information is obtained which suggest possible mechanisms of interfering with the infectious disease process, e.g., by means of specific antimetabolites, etc. Likely materials, obtained commercially and synthesized in this laboratory will be tested for virucidal activity.

B. Clinical Studies:

1. Physico-chemical fractionation of viruses. By means of physico-chemical techniques, e.g. electrophoresis, ion-exchange chromatography, metal-complexes, et . pathogenic viruses are being isolated from tissue culture fluids. This work is being extended so that these techniques may be applied to the detection of viral disease agents in any biologic material.

2. Cystic fibrosis of the pancreas. Because of the abnormally high concentration of sodium chloride in the sweat of patients with cystic fibrosis of the pancreas, other components of the sweat of such patients will be studied in the attempt to elucidate the underlying mechanism of this abnormality.

3. Serum iron study. One of the responses of the body to acute infection is a fall both in serum iron and in iron-binding capacity. To test the hypothesis that this is a result of adreno-cortical stimulation of the reticulo-endothelial system, resulting in an increased uptake of serum iron by the RE system, ACTH is being given to both normals and chronically ill patients and their serum iron levels are being followed. In-vitro studies on hepatic uptake of Fe^{59} in the presence of serum from chronically ill donors is planned.

Major Findings:

A. Basic Studies:

1. It had been established previously (see Progress Report NMI - 7c, 1955) that the growth-promoting activity of whole serum protein for mammalian cells in tissue culture was associated with one particular protein fraction. This fraction had the electrophoretic motility of an alpha$_2$ protein. Their growth-promoting ability of such a protein is quite unique and stands in marked contrast to the requirement for serum albumin by a number of diverse fastidious micro-organisms.

2. Most current media for the cultivation of cells in tissue culture utilize carbondioxide/sodium bicarbonate as the buffer system. This buffer system suffers from the rapid change in pH when such cultures are exposed to the atmosphere as the result of escape of CO_2. Conventional buffer systems (phosphate, citrate, etc.) cannot be used at the necessary levels because of their removal of essential cations from the culture medium by chelation and by precipitation. It has been found that phosphite can be used as an effective buffer without toxic effects, thus simplifying technical handling of tissue culture materials.

3. A crude enzyme preparation has been obtained from rat liver which catalyzes the oxidation of 1,2-propandio. This substance, as the phosphate, is a naturally occurring compound (brain, etc.) which has not yet been identified as an intermediate in carbohydrate metabolism despite its close relationship to several three-carbon key intermediates of carbohydrate metabolism. The primary product of the enzymatic oxidation of 1,2-propandiol appears to be lactic aldehyde. Lactic aldehyde, which has never been isolated as a biological intermediate, would then presumably be further oxidized to lactic acid, perhaps the rough methylglyoxal. The latter reactions are being investigated.

4. The APC family of viruses produce "soluble" antigens when grown in tissue culture. These antigens are specific for APC viruses but are heterotypic for individual strains when measured by complement fixation with APC anti-serum. Clinical diagnosis of infection with an APC virus is based on complement fixation by this "soluble" antigen. Each APC virus appears to produce but one such antigen which is smaller than and distinct from the infectious viral particle. Infective virus does not react per se in the CF test. Techniques have been found for isolating this complement fixing material. It is planned to characterize these CF antigens and determine their relationship to the growth cycle of the infective particle.

B. Clinical Studies:

1. The secretion of NaCl in the sweat of patients with cystic fibrosis of the pancreas has been determined under a number of conditions:
   a) various sites
   b) various flow rates
   c) various stimuli, e.g. thermal, pharmacologic
   d) various nutritional and metabolic conditions

   These data have been analyzed in an attempt to characterize the precursor material of sweat and to elucidate t ism of the abnormal ion transport that is found with th of such patients.

2. Intravenous ACTH was given to a number of normal volunteers and to several chronically ill patients. The effect on serum iron and on iron-binding-capacity was followed and compared with normal diurnal changes in these values. There was no consistent effect on serum iron as the result of treatment with ACTH.

Significance to Microbiological Research:

The biochemical studies outlined in this project represents a two-pronged attack on the diagnosis and treatment of infectious disease.

On the basic side, information is being gathered which is essential to the understanding of the biochemical effects produced by the infectious disease process. At the same time, clues are obtained which may be of use in counteracting and preventing these effects.

On the clinical side, the biochemical studies on fibrocystic disease of the pancreas illustrate the methodology used in attacking the problem of understanding a disease whose very etiology is unknown. Such studies are essential prerequisites for diagnosis and for therapy.

Proposed Course of Project:

Studies will continue as described above.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-18(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$40,475	4,526	45,001	2.6	2.0	4.6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3	3	6	46

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTE OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID - 18 (c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 19(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Leptospirosis
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. John P. Utz
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION

Objectives:

Studies are directed toward evaluation and improvement of methods of diagnosis, toward determining the clinical course and complications and sequellae due to the more recently recognized species of Leptospira, in particular L. pomona. Therapeutic agents will be evaluated.

11. Methods Employed:

Clinical study of patients; experimental therapy; laboratory diagnostic techniques, epidemiological surveys on occupational groups; etiologic surveys on patients with aseptic meningitis.

Major Findings:

None. Patient material not available.

Significance to Microbiological Research:

Leptospirosis is widespread in our domestic animals. The extent of the problem in man and reliability of diagnostic tests needs definition, as does the clinical spectrum of the disease. No effective therapy is known.

Proposed Course of Project:

If patient material becomes available, project will be activated.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-19(C)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	-	-	-	-	-	-

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	-	-	-	-

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards & Publications 15. NIAID - 19(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards & Publications 15. NIAID - 19(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 20(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Q fever
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION:

Objectives:

To evaluate Q fever as a cause of chronic disease; to study chemotherapy on patients with presumed residua of previous illness; to study basic immunology of long term complement-fixation antibody levels.

Methods Employed:

Hospitalization of acutely ill patients who have had chronic complaints post-infection; isolation attempts using animal, chick embryo and tissue culture techniques; clinical observation with laboratory studies of chemotherapy response.

Major Findings:

A few additional patients have been hospitalized and carefully studied. A yet unevaluated finding in two such patients was the presence of pericarditis clinically. An additional patient presented with monoarticular arthritis of the knee for which there was no apparent cause other than old infection altho this relationship was not proved by culture of the organism.

Significance to Microbiological Research:

All patients studied thus far in this project are NIH employees, some of whom are on disability retirement. The results of these studies should have application to the general field of rickettsial illness, some of which are important causes for military service disability.

Proposed Course of Project:

This project is temporarily inactivated due to lack of patient material. If patients become available, the project will be reactivated.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 20(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Q fever
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

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Major Findings:

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Proposed Course of Project:

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Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 20(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Q fever
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION:

Objectives:

To evaluate Q fever as a cause of chronic disease; to study chemotherapy on patients with presumed residua of previous illness; to study basic immunology of long term complement-fixation antibody levels.

Methods Employed:

Hospitalization of acutely ill patients who have had chronic complaints post-infection; isolation attempts using animal, chick embryo and tissue culture techniques; clinical observation with laboratory studies of chemotherapy response.

Major Findings:

A few additional patients have been hospitalized and carefully studied. A yet unevaluated finding in two such patients was the presence of pericarditis clinically. An additional patient presented with monoarticular arthritis of the knee for which there was no apparent cause other than old infection altho this relationship was not proved by culture of the organism.

Significance to Microbiological Research:

All patients studied thus far in this project are NIH employees, some of whom are on disability retirement. The results of these studies should have application to the general field of rickettsial illness, some of which are important causes for military service disability.

Proposed Course of Project:

This project is temporarily inactivated due to lack of patient material. If patients become available, the project will be reactivated.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 20(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Q fever
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION:

Objectives:

To evaluate Q fever as a cause of chronic disease; to study chemotherapy on patients with presumed residua of previous illness; to study basic immunology of long term complement-fixation antibody levels.

Methods Employed:

Hospitalization of acutely ill patients who have had chronic complaints post-infection; isolation attempts using animal, chick embryo and tissue culture techniques; clinical observation with laboratory studies of chemotherapy response.

Major Findings:

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Significance to Microbiological Research:

All patients studied thus far in this project are NIH employees, some of whom are on disability retirement. The results of these studies should have application to the general field of rickettsial illness, some of which are important causes for military service disability.

Proposed Course of Project:

This project is temporarily inactivated due to lack of patient material. If patients become available, the project will be reactivated.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 21(c)
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Clinical Investigation
LABORATORY, BRANCH, OR DEPARTMENT

4. None
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Immunochemistry
PROJECT TITLE

7. Dr. Sheldon Gray
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

10. PROJECT DESCRIPTION:

Objectives:

The general objective of this project is to contribute to the understanding of immunologic and allergic phenomena in terms of physical and chemical reactions. More specifically, the following problems will be undertaken:

1. To develop methods for the quantitative determination of non-precipitating antibodies such as found in allergic diseases.

2. To extend the existing methods for the quantitative determination of the antigenic components of human tissues.

3. To develop a well-defined system, possibly by the use of tissue culture, for the study of the biosynthesis of antibodies.

4. To study the mechanism of tissue damage resulting from antigen-antibody reactions.

5. To develop better methods for the isolation and purification of the specific antigens in various pollens and microorganisms.

Methods Employed:

1. The gel diffusion method for the immunochemical analysis of antigen-antibody reactions.

2. Chemical analysis of antigen-antibody precipitates.

3. Freunds adjuvant technique for the immunization of rabbits.

4. Prausnitz-Kustner passive transfer technique for the determination of reagin.

Major Findings:

1. Rabbit precipitins to rabbit serum proteins have been produced by immunization procedures involving the use of immune precipitates and adjuvants. The results partially confirm the work of Oudin who originally described this phenomenon. The results also yielded new information currently under further investigation.

2. Rabbits were immunized with ragweed pollen to produce antisera for the study of various fractions of ragweed pollen by the gel diffusion techniques. Preliminary results of the fractionation of ragweed pollen by ion-exchange chromatography indicate that this may be a feasible method for the preparation of an immunochemically distinct allergen from ragweed pollen.

3. Studies on human serum proteins have been started with the preparation of antisera to highly purified preparations of human gamma-globulin and albumin.

Significance to Microbiological Research:

St Studies in immunochemistry should lead to a better understanding of the pathogenesis of allergic and infectious diseases and thereby provide a basis for better clinical management of these diseases.

Proposed Course of Project:

As described.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-21(c)
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$11,855		$11,855	1.0		1.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1		1	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards and Publications

15. NIAID - 21(c)
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 36
SERIAL NUMBER

2. Allergy & Infectious Diseases 3. Laboratory of Infectious Diseases

4. Office of the Chief, LID 5. _____
SECTION OR SERVICE LOCATION (IF OTHER THAN BETHESDA

6. Office of the Chief, LID
PROJECT TITLE

7. Dr. Robert J. Huebner (Chief)

8. Dr. Roger M. Cole (Asst. Chief); Jeni Arliss (Adm. Asst.)
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

Office of the Chief.

Objectives:

Since top-flight, original research can only be the product of the individual scientist or a team of scientists, the principal aim and function of the Chief is, 1) to recognize and to assist in the selection and development of top-flight scientific workers, and 2) to provide opportunities for them to achieve maximum effectiveness and productivity. Support of program with respect to content, is conceived to be of equal importance, but the extent of support is regarded as dependent upon promise or established caliber of the investigators available and, of course, upon their interests and their areas of competence.

Changes in Program and Personnel:

Dr. R. J. Huebner replaced Dr. Dorland J. Davis as Chief

of the Laboratory of Infectious Diseases. Dr. Roger Cole was appointed to the newly-graded position of Assistant Chief. Two young investigators in the Virus Section, after several years of solid accomplishment as members of team research efforts, were regarded as qualified to head up independent research units. Steps were, therefore, taken to correct an imbalance between opportunities in LID for viral and bacteriological research -- the latter having several times the floor space available to the Virus Section. Dr. Wallace P. Rowe was placed in charge of a new research unit in the Virus Section, replacing the Brucellosis Unit of the Bacteriology Section, which unit, in turn, was transferred to the Laboratory of Clinical Investigations. Dr. Alexis Shelokov, formerly associated with Dr. Habel in the Basic Studies Section, was placed in charge of the Laboratory Unit (formerly Influenza Unit) of the Epidemiology Section. Dr. Kenneth Takemoto (Laboratory Unit, Epidemiology Section) was transferred to the Basic Studies Section. Dr. Leon Rosen, Laboratory of Tropical Diseases, was reassigned to the Virus Section of LID.

Administration:

In order to serve the general best interests of effective research, the administrative office of LID was strengthened with additional clerical help. Greater responsibility was placed upon the position of Assistant Chief, which job was graded at the Sr. Surgeon level. Similarly, considerable responsibility for developing a more businesslike administration of the laboratory operation was placed on the Administrative Assistant, Mrs. Jeni Arliss

Form No. OMP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-36

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$58,400	21,000	79,400	0.4	8.2	8.6

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	9	10

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

PART C: Honors, Awards & Publications 15. NIAID - 36
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Robert J. Huebner:

General Chairman of the Conference on "Viruses in Search of Disease" sponsored by the New York Academy of Sciences, May 24-25, 1956.

Awarded gold plaque at the Brooklyn College of Pharmacy's Annual Public Health Forum, March 13, 1956.

The Benjamin Knox Rachford Lecturer - Children's Hospital Research Foundation, Cincinnati, Ohio - Feb. 14-15, 1956.

Form No. ORF-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 37
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Experimental Therapeutics
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN B. THESDA)

6. Nutrition and Metabolism of Normal and Malignant Cells in Tissue Culture
PROJECT TITLE

7. Dr. Harry Eagle
PRINCIPAL INVESTIGATOR

8. Dr. Stanley Barban, Dr. James Darnell, Mr. Vance Oyama, Mr. Ralph Fleischman, Miss Nina Levy, Mr. Aaron Freeman, and Mr. Henry Schulze
OTHER INVESTIGATORS

9. Dr. George Foley, Harvard Medical School, Boston, Massachusetts.

10. PROJECT DESCRIPTION

a. Carbohydrate metabolism in tissue culture (Eagle, Barban, Schulze, Levy)

Objectives:

To determine the carbohydrates which can be utilized by normal and malignant cells in culture and the pathways involved in their metabolism.

Major Findings:

A number of carbohydrates other than glucose have been found to substitute for the latter in tissue culture (fructose, galactose, maltose, mannose, trehalose, turanose). There were wide differences in the amounts of acid produced on these varying substrates. In both respects, there were no significant differences between normal and malignant cells.

Significance to NIAID Research:

An understanding of the physiologic processes involved in carbohydrate utilization is of broad biological significance, and has implications also with respect to the mechanisms involved in virus synthesis.

Proposed Course of Project:

(1) To determine the degree to which these other sugars can substitute for glucose in biosynthetic reactions for which glucose is normally employed, and in particular in the formation of virus.

(2) To explore the metabolic pathways involved in the utilization of these various "non-physiological" sugars.

b. Amino acid metabolism and protein synthesis in tissue culture (Eagle, Levintow, Piez, Fleischman)

Objectives:

To study the metabolic pathways by which amino acids are incorporated into protein, and the degree to which they are interconverted.

Major Findings:

(1) (a) Glutamine has been shown to be an essential building block for the synthesis of protein by the HeLa cell in tissue culture. Studies with glutamine doubly labeled with C^{14} and N^{15} have revealed that it is incorporated into the protein as such without preliminary degradation. It is therefore an essential amino acid for these cells in the usually recognized sense.

(b) An incidental finding of some importance was the fact that the glutamine in the medium of cells in tissue culture is rapidly broken down to glutamic acid and ammonia. The glutamic acid residues of the cell protein were shown to be derived from the glutamic acid thus formed in the medium.

(c) In the course of these studies a convenient method for the synthesis of L-glutamine labeled with radioactive or stable isotopes has been developed. Another technique which may prove to be of general usefulness is a procedure for the separate isolation of glutamine, glutamic acid, asparagine, and aspartic acid from small amounts of protein.

(d) An interesting and unexpected byproduct of these studies has been the finding that C^{14}-labeled amino acids differ in their ion exchange behavior from the corresponding unlabeled compounds. This resolution appears to depend on an effect of the C^{14} atom on the dissociation of an adjoining NH_3^+ or COO^- group.

(2) Studies with isotopically labeled phenylalanine, tyrosine, histidine and arginine have shown that these two amino acids are used primarily for incorporation into protein, and are not metabolized by the cell to any important degree. A variant of the HeLa cell has, however, been found which to a limited extent converts phenylalanine to tyrosine.

(3) A byproduct of these studies has been the interesting and important finding that there is apparently a dynamic equilibrium between the cell proteins and the intracellular amino acid pool. The formed proteins are apparently constantly being broken down and resynthesized. This intracellular protein turnover, postulated by Schönheimer, has not been found in bacterial cells.

Significance to NIAID Research:

The physiology of mammalian cells has obvious broad implications, including the processes of inflammation and viral propagation.

Proposed Course of Project:

(1) The mechanism by which the cells degrade glutamine and the significance of this phenomenon in the metabolism of the cells will be investigated.

(2) The only component, other than glutamine, of the HeLa cell protein which appears to utilize the amide nitrogen of glutamine is asparagine. This clue to the hitherto unknown course of asparagine biosynthesis will be investigated.

(3) Studies will be continued with other amino acids to determine the degree to which they are used simply for incorporation into protein, or can be degraded by the cell to smaller fractions which can then be used for energetic metabolism or biosynthesis.

(4) An attempt will be made to determine the metabolic block which prevents the cells from synthesizing the nutritionally essential amino acids. Conversely, attempts will be made to determine the major pathways for the biosynthesis of the amino acids which the cell does not require for growth.

c. The effect of carcinolytic agents on normal and malignant cells in tissue culture (Eagle, Foley, Freeman)

Objectives:

To determine the effects of carcinolytic agents on normal and malignant cells in tissue culture.

Major Findings:

A wide variety of anti-tumor agents have been found to have a high degree of cytotoxicity in tissue culture, so high indeed as to suggest that this direct cytotoxicity may be the basis for their anti-tumor action in vivo. Under such circumstances, the slight selectivity which these compounds demonstrate in vivo would be due to host-imposed differences not demonstrable in culture.

Proposed Course of Project:

(1) To determine the degree to which these other sugars can substitute for glucose in biosynthetic reactions for which glucose is normally employed, and in particular in the formation of virus.

(2) To explore the metabolic pathways involved in the utilization of these various "non-physiological" sugars.

b. Amino acid metabolism and protein synthesis in tissue culture (Eagle, Levintow, Piez, Fleischman)

Objectives:

To study the metabolic pathways by which amino acids are incorporated into protein, and the degree to which they are interconverted.

Major Findings:

(1) (a) Glutamine has been shown to be an essential building block for the synthesis of protein by the HeLa cell in tissue culture. Studies with glutamine doubly labeled with C^{14} and N^{15} have revealed that it is incorporated into the protein as such without preliminary degradation. It is therefore an essential amino acid for these cells in the usually recognized sense.

(b) An incidental finding of some importance was the fact that the glutamine in the medium of cells in tissue culture is rapidly broken down to glutamic acid and ammonia. The glutamic acid residues of the cell protein were shown to be derived from the glutamic acid thus formed in the medium.

(c) In the course of these studies a convenient method for the synthesis of L-glutamine labeled with radioactive or stable isotopes has been developed. Another technique which may prove to be of general usefulness is a procedure for the separate isolation of glutamine, glutamic acid, asparagine, and aspartic acid from small amounts of protein.

(d) An interesting and unexpected byproduct of these studies has been the finding that C^{14}-labeled amino acids differ in their ion exchange behavior from the corresponding unlabeled compounds. This resolution appears to depend on an effect of the C^{14} atom on the dissociation of an adjoining NH_3^+ or COO^- group.

(2) Studies with isotopically labeled phenylalanine, tyrosine, histidine and arginine have shown that these two amino acids are used primarily for incorporation into protein, and are not metabolized by the cell to any important degree. A variant of the HeLa cell has, however, been found which to a limited extent converts phenylalanine to tyrosine.

(3) A byproduct of these studies has been the interesting and important finding that there is apparently a dynamic equilibrium between the cell proteins and the intracellular amino acid pool. The formed proteins are apparently constantly being broken down and resynthesized. This intracellular protein turnover, postulated by Schönheimer, has not been found in bacterial cells.

Significance to NIAID Research:

The physiology of mammalian cells has obvious broad implications, including the processes of inflammation and viral propagation.

Proposed Course of Project:

(1) The mechanism by which the cells degrade glutamine and the significance of this phenomenon in the metabolism of the cells will be investigated.

(2) The only component, other than glutamine, of the HeLa cell protein which appears to utilize the amide nitrogen of glutamine is asparagine. This clue to the hitherto unknown course of asparagine biosynthesis will be investigated.

(3) Studies will be continued with other amino acids to determine the degree to which they are used simply for incorporation into protein, or can be degraded by the cell to smaller fractions which can then be used for energetic metabolism or biosynthesis.

(4) An attempt will be made to determine the metabolic block which prevents the cells from synthesizing the nutritionally essential amino acids. Conversely, attempts will be made to determine the major pathways for the biosynthesis of the amino acids which the cell does not require for growth.

c. The effect of carcinolytic agents on normal and malignant cells in tissue culture (Eagle, Foley, Freeman)

Objectives:

To determine the effects of carcinolytic agents on normal and malignant cells in tissue culture.

Major Findings:

A wide variety of anti-tumor agents have been found to have a high degree of cytotoxicity in tissue culture, so high indeed as to suggest that this direct cytotoxicity may be the basis for their anti-tumor action in vivo. Under such circumstances, the slight selectivity which these compounds demonstrate in vivo would be due to host-imposed differences not demonstrable in culture.

Significance to NIAID Research:

These findings are primarily of interest in relation to the mechanism of action of anti-tumor agents.

Proposed Course of Project:

Studies are in progress with a larger series of compounds to confirm and extend the observations already made. In particular, an attempt will be made to determine whether tissue culture toxicity can be used as a primary screen for the detection of anti-tumor agents. If it should indeed prove that the large majority of compounds known to have an effect on tumor are highly cytotoxic in tissue culture and if, conversely, a relatively small proportion of compounds devoid of anti-tumor activity are toxic to the same degree, a simple method becomes available for the screening of large numbers of compounds for potential anti-tumor action.

d. Virus production in tissue culture (Eagle, Darnell, Habel)

Objectives:

(1) Nutritional requirements for production of various viruses in HeLa, KB, and mouse fibroblasts.

(2) Explore by radioisotopic techniques the pathways of viral biosynthesis.

Major Findings:

Previous findings indicated that glucose, glutamine and salts were both necessary and sufficient for the production of type II polio virus by HeLa cells. This appears also to be true for other types of polio as well as for EMC and APC viruses. These metabolites may, however, be insufficient for the optimum multiplication of larger viruses such as vaccinia and herpes simplex.

Significance to NIAID Research:

These studies provide an approach to the nutritional requirements for the propagation of viruses, the metabolic processes involved in their biosynthesis, and their composition.

Proposed Course of Project:

(1) The determination of the nutritional requirements for the biosynthesis of virus will be extended to other cell-virus systems.

(2) Viral assay by plaque-counting techniques should facilitate these experiments. Purification of virus by either adsorption-elution from red blood cells, or a simplified crystallization procedure, should allow attempts at the incorporation of isotopically labeled precursors in order to determine metabolic pathways involved in biosynthesis.

(3) Experiments are in progress to determine the degree to which vitamins are necessary for virus synthesis.

e. The nutritional requirements for mammalian cells in tissue culture (Eagle, Oyama).

Objectives:

To define completely the nutritional requirements for the sustained propagation of animal cells in tissue culture.

Major Findings:

The amino acid, vitamin, carbohydrate and salt requirements have been determined for a variety of normal and malignant cells in tissue culture. A small amount of serum protein must, however, be added to these essential components in order to permit growth; and the nutritional function of the serum protein remains to be determined. There is now reason to believe that the serum protein contributes a number of small molecular weight components which are merely bound to the protein and that this is indeed its sole function.

Significance to NIAID Research:

The physiology of mammalian cells has obvious broad implications, including the processes of inflammation and viral propagation.

Proposed Course of Project:

(1) The paradoxic requirements for 13 amino acids, as contrasted with the 8 amino acids required by the human host, and the limited requirement for vitamins, suggested that the findings to date might be an artefact, relating to cells which have been serially propagated in tissue culture for years, and which no longer resemble cells in the living host. Accordingly, the amino acid and vitamin requirements are being determined for a number of recently isolated cell lines, as well as for cells directly isolated from the animal host and in their first tissue culture passage.

(2) Attempts are continuing to determine the nature of the substances supplied by serum protein.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-37

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	97,351	56,552	153,903	4.0	7.0	11.0

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	4	7	11

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14 IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. Leon Levintow, NCI
Dr. Karl Piez, NIDR

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards - Publications 15. NIAID-37
SPECIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

a. None.

b. Eagle, H., and Piez, K.A. C^{14} isotope effect on the ion-exchange chromatography of amino acids. J. Am. Chem. Soc., 78: 5284-7, 1956.

c. Eagle, H., and Foley, G.E. The cytotoxic action of carcinolytic agents in tissue culture. Am. J. Med., 21: 739-749, 1956.

d. Eagle, H., Habel, K., Rowe, W.P., and Huebner, R.J. Viral susceptibility of a human carcinoma cell (strain HE). Proc. Soc. Exp. Biol. Med., 91: 361-4, 1956.

Eagle, H., and Habel, K. The nutritional requirements for the propagation of poliomyelitis virus by the HeLa cell. J. Exp. Med., 104: 271-287, 1956.

e. Oyama, V.I., and Eagle, H. Measurement of cell growth in tissue culture with a phenol reagent (Folin-Ciocalteau). Proc. Soc. Exp. Biol. Med., 91: 305-7, 1956.

Eagle, H. Relative growth-promoting activity in tissue culture of co-factors and the parent vitamins. Proc. Soc. Exp. Biol. Med., 91: 358-361, 1956.

Eagle, H., Oyama, V.I., Levy, M., and Freeman, A. Myo-inositol as an essential growth factor for normal and malignant human cells in tissue culture. Science, 123: 845-7, 1956.

Eagle, H., Oyama, V.I., Levy, M., and Freeman, A. Myo-inositol as an essential growth factor for normal and malignant human cells in tissue culture. J. Biol. Chem. (in press).

Eagle, H., Oyama, V.I., and Levy, M. Amino acid requirements of normal and malignant human cells in tissue culture. Arch. Biochem. & Biophys. (in press).

17. List honors and awards to personnel relating to this project during calendar year 1956:

None.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-38
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Experimental Therapeutics
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. In Vitro Synthesis of Antibodies
PROJECT TITLE

7. Dr. Arthur K. Saz
PRINCIPAL INVESTIGATOR

8. Mrs. Marina Martinez, Mr. Moses Middleton
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objective: To study the in vitro synthesis of antibodies.

Major Finding:

Cells derived from the spleens of non-immunized rabbits are at present being cultured.

Significance to NIAID Research:

The mechanism of antibody synthesis is one of the major unsolved problems of immunology. In addition, since antibodies are proteins, and are marked as to biological activity, this study is ideally suited for procuring information concerning the pathways of protein synthesis.

Proposed Course of Project:

Tissue culture will be attempted of presumed antibody-producing organs, and particularly of spleen, from both non-immunized and previously immunized rabbits. Exposures of these tissue cells to antigens and assay of both the cells and culture fluid for antibody synthesis will be carried out. Antibody formation will be followed by a variety of techniques, including the in vitro hemagglutination technique of Boyden.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-38

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$12,093	7,046	19,139	0.5	0.8	1.3

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	1	2

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-38
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956

None

Form No. CRP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID-39
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Experimental Therapeutics
SECTION OR SERVICE

5.
LOCATION (If Other Than Bethesda)

6. Mode of Action of Aureomycin
PROJECT TITLE

7. Dr. Arthur K. Saz
PRINCIPAL INVESTIGATOR

8. Mrs. Marina Martinez, Mr. Moses Tidwell
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To determine the biochemical basis for the inhibition of sensitive bacteria by aureomycin, and the biochemical mechanism of resistance of bacteria to the antibiotic. To determine also why aureomycin at clinically used concentrations is bacteriostatic to sensitive cells, rather than bactericidal.

Methods:

Isolation, resolution and purification of various electron transport enzymes from both sensitive and resistant E. coli will be carried out.

Major findings

It had previously been found that cell-free nitro reductase, and various other electron transport enzymatic systems (including, ferricyanide and phenol-indophenol reductases) are flavoproteins. These enzymes, when derived from sensitive cells, are sensitive to aureomycin, while similar enzymes derived from resistant cells are resistant to the antibiotic. Both sensitive and resistant enzymes are metalloflavoproteins, the former apparently a manganoenzyme with an

present in concentrations less than 10^{-8} moles per gram protein. The resistant enzyme probably is a molybdoflavoprotein with extremely low, though tenaciously bound, Mo. Electron transport enzymes of two types, apparently mediating similar functions, have been isolated from highly purified extracts of the sensitive cell. One enzyme is extremely sensitive to aureomycin, the other is quite resistant. If both these enzymes are functionally active in the cell, this observation would provide a possible explanation for the bacteriostatic rather than bactericidal activity of the antibiotic, since the resistant enzyme may partially compensate for that inhibited by the antibiotic. Extracts from the resistant cell contain apparently only one type of enzyme, and this is resistant to the antibiotic.

Significance to NIAID Research:

Studies on the mode of action of antibiotics, and on the mechanisms involved in the development of resistance to these compounds, provide a rational approach to the chemotherapy of infectious disease.

Proposed Course of Project:

It is proposed to continue studying the electron transport enzyme systems of sensitive and resistant cells to determine more precisely the biochemical differences between the two.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID-39
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Experimental Therapeutics
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Mode of Action of Aureomycin
PROJECT TITLE

7. Dr. Arthur K. Saz
PRINCIPAL INVESTIGATOR

8. Mrs. Marina Martinez, Mr. Moses Middleton
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To determine the biochemical basis for the inhibition of sensitive bacteria by aureomycin, and the biochemical mechanism of resistance of bacteria to the antibiotic. To determine also why aureomycin at clinically used concentrations is bacteriostatic to sensitive cells, rather than bactericidal.

Methods:

Isolation, resolution and purification of various electron transport enzymes from both sensitive and resistant E. coli will be carried out.

Major Findings

It had previously been found that cell-free nitro reductase, and various other electron transport enzymatic systems (including ferricyanide and phenol-indophenol reductases) are flavoproteins. These enzymes, when derived from sensitive cells, are sensitive to aureomycin, while similar enzymes derived from resistant cells are resistant to the antibiotic. Both sensitive and resistant enzymes are metalloflavoproteins, the former apparently a mangano enzyme with Mn

present in concentrations less than 10^{-8} moles per gram protein. The resistant enzyme probably is a molybdoflavoprotein with extremely low, though tenaciously bound, Mo. Electron transport enzymes of two types, apparently mediating similar functions, have been isolated from highly purified extracts of the sensitive cell. One enzyme is extremely sensitive to aureomycin, the other is quite resistant. If both these enzymes are functionally active in the cell, this observation would provide a possible explanation for the bacteriostatic rather than bactericidal activity of the antibiotic, since the resistant enzyme may partially compensate for that inhibited by the antibiotic. Extracts from the resistant cell contain apparently only one type of enzyme, and this is resistant to the antibiotic.

Significance to NIAID Research:

Studies on the mode of action of antibiotics, and on the mechanisms involved in the development of resistance to these compounds, provide a rational approach to the chemotherapy of infectious disease.

Proposed Course of Project:

It is proposed to continue studying the electron transport enzyme systems of sensitive and resistant cells to determine more precisely the biochemical differences between the two.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-39
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Saz, A.K., and Martinez, L.M. Enzymatic basis of resistance to aureomycin. 1. Differences between flavoprotein nitro reductases of sensitive and resistant Escherichia coli. J. Biol. Chem. (in press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-40
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Experimental Therapeutics
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Nucleic Acid Synthesis by Mammalian Cells in Tissue Culture
PROJECT TITLE

7. Dr. Norman P. Salzman
PRINCIPAL INVESTIGATOR

8. Dr. Harry Eagle, Mr. Edwin Sebring
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION:

Objectives:

To elucidate the pathways by which ribonucleic acid (RNA) and deoxynucleic acid (DNA) are synthesized by human cells in tissue culture, and the effect of viral infection on nucleic acid biosynthesis.

Major Findings:

(1) Glutamine

Preliminary incorporation studies with glutamine-2-C^{14} amide N^{15} suggested that the efficient utilization of the carbon skeleton for pyrimidine synthesis depended initially upon the conversion of glutamine to glutamic acid. The latter compound is then converted via the tricarboxylic acid cycle and transamination to aspartic acid, a known pyrimidine precursor in other mammalian systems. This was confirmed when HeLa cells grown in a medium containing non-radioactive glutamine and radioactive glutamic acid showed the same pattern of labeling as had been obtained with radioactive glutamine in the absence of glutamic acid.

The amide N of glutamine has been shown to be extensively incorporated into both the purines and pyrimidines of RNA and DNA. When cells are grown in a minimal medium embodying only those factors essential for growth, a high level of glutamine is present (1.5 mM), but no NH_4^+ is added to the medium. The rapid breakdown of glutamine to glutamic acid with the liberation of NH_4^+ is the source of the ammonia pool. RNA guanine and adenine have been isolated from cells grown in a medium containing glutamine-amide N^{15} and by treatment with nitrous acid converted to xanthine and hypoxanthine, respectively. These studies indicate that the guanine amino group is not derived from glutamine or ammonia as has been reported for other systems. This points to what may prove to be important differences between HeLa and other cell systems.

(2) The utilization of guanine-C^{14}, adenine-8-C^{14} and glycine-C^{14} for RNA and DNA synthesis has been studied. In addition, histidine-7-8-N^{15}-2-C^{14} was synthesized and its incorporation studied. These experiments are presently being completed.

Significance to NIAID Research:

Nucleic acid biosynthesis is an essential aspect of viral propagation; and an understanding of that process in the infected cell is obviously basic to an understanding of the course of events after viral infection.

Proposed Course of Project:

(1) The present study will be extended to include an examination of RNA and DNA turnover in HeLa cells.

(2) The role of glutamine in nucleic acid biosynthesis will be further studied.

(3) The effect of viruses on nucleic acid synthesis will be studied, using appropriate labeled precursors.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-40

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$21,688	12,607	34,295	1.3	1.3	2.6

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	2	3

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. OR-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-40
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956.

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 41
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Experimental Therapeutics
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Metabolic Pathways of Cells in Tissue Culture
PROJECT TITLE

7. Dr. Stanley Barban
PRINCIPAL INVESTIGATOR

8. Mr. Henry Schulze
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To study the metabolic patterns of normal and malignant mammalian cells in tissue culture.

Major Findings:

Continuation of a comparative study on the scope of amino acid interconversions in normal and malignant cells in tissue culture has revealed some notable quantitative differences in the cell lines. Certain amino acids, particularly glutamic, aspartic, isoleucine, leucine, and alanine, were most active as amino donors in transamination reaction. Other amino acids were relatively weak or inert as amino donors. Exploration of the possibility that L-glutamine, an essential amino acid, might function as an amino donor to some α-keto acids was undertaken. It was found that it was an active amino donor, but not as active as L-glutamic acid in various transamination reactions.

Significance to NIAID Research:

(1) Study of the metabolic pathways of tissue cells grown in tissue culture provides an excellent opportunity for investigating the

(2) Studies on transamination are important in affording an approach to the biosynthesis and degradation of amino acids by mammalian tissue cells.

Proposed Course of Project:

To continue the comparative study on the scope of transamination reactions employing different cell lines, both normal and malignant, with particular emphasis on specificity of certain amino acids, and to explore the notable differences found.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-41

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,022	8,714	23,736	0.8	1.0	1.8

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	1	2

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID - 41
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Barban, S., and Schulze, H.O. Metabolism of tissue culture cells. The presence in HeLa cells of the enzymes of the citric acid cycle. J. Biol. Chem., 222: 665-670, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-42
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Experimental Therapeutics
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Genetic variation in cultures of mammalian cells
PROJECT TITLE

7. Dr. Robert DeMars
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To prepare an assortment of mutants that might be used in studying the behavior of human genetic material in vitro.

Major Findings:

(1) HeLa cells require glutamine in the medium in order to survive and grow. We are looking for glutamine-independent mutants. Prolonged glutamine deprivation results in the disappearance, in about 8 weeks, of all the cells in cultures consisting of up to 2×10^9 cells. In three trials, X-irradiation of the cultures (100r to 800r) prior to starvation has resulted in the persistence of large clumps of cells after 8 weeks. These clumps arise by cell proliferation while most of the cells in the culture are dying. Cells isolated from these clumps have not yet given evidence of extensive proliferation in the absence of glutamine. They do, however, survive as viable cells much longer than do cells of the parent culture in the absence of glutamine. We are attempting to propagate them in specially supplemented media that reduce but do not abolish the glutamine requirement of the parent type cells.

(2) We are looking for poliomyelitis-resistant mutants of HeLa. Polio II was used to infect about 10^8 X-irradiated (150r) HeLa cells. After 24 hours very few cells remained on the walls of the culture vessels. These were covered with an agar-containing medium which promotes colony formation by single cells. Any clones which appear

Clones surviving this treatment will almost certainly be pure cultures of polio-resistant cells.

Proposed course of project:

(1) To select for other types of mutants.

(2) To select one or two of these that occur frequently and use them in standardizing methods of inducing and selecting for mutations.

(3) To devise a system, using these mutants, for studying genetic recombination in human cells in vitro.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-42

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	1,333	742	2,075	-	0.3	0.3

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	4	-	-

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-42
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-43
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Basic studies of virus-host cell relationships.
PROJECT TITLE

7. Dr. Karl Habel
PRINCIPAL INVESTIGATOR

8. Dr. Kenneth K. Takemoto and Nancy C. Gregg
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To determine factors important to the invasion, multiplication and release of virus from an animal host cell.

Methods Employed:

Various virus-tissue culture cell systems are being investigated from the standpoint of the effect of medium deficiencies on the virus multiplication. Latent infections of cells will be established and studied for the factors responsible for viral latency and its unmasking.

Major Findings:

With Dr. Eagle of NIAID we have demonstrated that glucose and glutamine in the tissue culture medium are essential for poliovirus multiplication in HeLa cells but not in freshly grown monkey kidney cells. The glucose-glutamine requirement appears to apply to other viruses in other established tissue culture cell lines.

Significance to the program of the Institute:

The intimate relation which viruses appear to have with the basic genetic processes of the cell make basic information concerning these relationships of broad biological significance.

Proposed Course of the Project:

These studies will continue with more interest in the latent virus infections.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-43

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	20,827	9,232	30,059	0.8	2.1	2.9

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	2	3

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. D. W. McBride, NIDR

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-43
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Viral Susceptibility of a Human Carcinoma Cell (Strain KB). Harry Eagle, Karl Habel, Wallace P. Rowe and Robert J. Huebner. Proc. Soc. Expl. Biol. & Med., 91(3):361-364, March 1956.

Nutritional requirements for propagation of poliomyelitis virus by the HeLa cell. Harry Eagle and Karl Habel. J. Exp. Med., 104(2):271-287, August 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

List of Publications not Related Entirely to this Project.

Effect on Immunity to Challenge and Antibody Response on Variation in Dosage Schedule of Rabies Vaccine in Mice. Karl Habel. Bull. Wld. Hlth. Org., 14:613-616, 1956.

Rabies Post-exposure Antibody Response to Different Schedules of Serum and Vaccine Inoculations in Non-exposed Persons. T. Atanasiu, M. Bahmoyar, M. DeLazzard, J. P. Fox, K. Habel, H. H. Kaplan, R. E. Kissling, A. Lorrov, H. Loprowski, P. Lepine, F. Perez-Gallardo. Bull. Wld. Hlth Org., 14:593-611, 1956.

Rabies (Hydrophobia). Karl Habel. Current Therapy. 1956.

Rabies Prophylaxis in Man. Karl Habel. Accepted by Pediatrics.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-44
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Evaluation of the clinical types of poliomyelitis from etiological point of view.
PROJECT TITLE

7. Dr. Karl Habel
PRINCIPAL INVESTIGATOR

8. Rosalie J. Silverberg and Nancy C. Gregg
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To determine if polioviruses are really the cause of all clinical disease diagnosed as poliomyelitis and to investigate factors which determine the severity of the clinical disease.

Methods Employed:

Isolation and identification of viral agents from clinically ill and control patients to establish etiological relationship of certain viruses other than polio with polio-like disease. Laboratory investigation of viruses for virulence in experimental animals.

Major Findings:

From materials collected in 1952, final identification of isolated viruses from nonparalytic polio and normal controls indicates that several of the B Coxsackie viruses and Echo 6 virus can cause a disease clinically identical with nonparalytic polio.

Major Findings (continued)

Investigations of a virus brought to us by Russian virologists and supposedly a "fourth type of polio" have shown it to be Coxsackie A7. However, this virus has been shown to be capable of producing paralysis and polio-like lesions in monkeys.

One hundred and four isolations of type 2 poliovirus have been evaluated for their virulence in mice and tissue culture. There is a tendency for viruses isolated from cases showing involvement of their central nervous system to be more virulent for mice than those isolates from cases of minor illness or healthy carriers.

Significance to the Program of the Institute:

Our findings, together with those of others, clearly point to the multiple viral etiology of so-called nonparalytic poliomyelitis. The Russian claim of isolating a fourth type of poliovirus has been disproven. Evidence has been brought forth to indicate that the poliovirus in patients with CNS involvement may differ from that in healthy carriers.

Proposed Course of the Project:

Most studies under this project have been completed. However, further studies are planned on investigating the influence of host cellular factors on the degree of virulence of poliovirus.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-44

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$22,674	10,032	32,706	0.8	2.4	3.2

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	2	3

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. C. P. Li, DBS

(Use reverse and additional pages, if necessary)

Form ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-44
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Subclinical Poliomyelitis in a Newborn Infant due to Intrauterine Infection. Alexis Shelokov and Karl Habel. J.A.M.A., 160(6):465-466 (Feb. 11, 1956).

Isolation of Enteric Viruses from Cases of Aseptic Meningitis. Karl Habel, Rosalie J. Silverberg and Alexis Shelokov. Accepted by the Annals of the New York Academy of Sciences.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID-45
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases (INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section SECTION OR SERVICE

5. Bethesda LOCATION (IF OTHER THAN BETHESDA)

6. Epidemiological and laboratory investigations of infectious diarrhea of infancy.
PROJECT TITLE

7. Dr. Karl Habel
PRINCIPAL INVESTIGATOR

8. Rosalie J. Silverberg and Dr. Kenneth K. Takemoto
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To determine the etiological agent or agents responsible for outbreaks of infectious diarrhea in young children.

Methods Employed:

A small orphanage with a population of 60 children under the age of 4 years in Cincinnati, Ohio, is under continuous surveillance by a nurse and physician who investigate the clinical and epidemiological aspects of any infectious disease occurring in the population. Every 2 weeks throat and rectal swabs are obtained on all children. Throat and rectal swabs are used to inoculate monkey kidney tissue cultures in an attempt to isolate viral agents. Rectal swabs are cultured bacteriologically for enteric pathogens.

Major Findings:

This project was initiated in July 1956. To date no definite outbreaks of infectious disease have occurred in the study population. No enteric pathogens have been isolated bacteriologically but frequent and persistent infections with viral agents have been demonstrated. Of 150 or more virus isolations to date, Coxsackie B3 and mostly Echo 7 have been identified, with no apparent correlation with a specific clinical illness.

Significance to the Program of the Institute:

The establishment of etiological relationship of viruses or bacteria with certain clinical infections must involve a study of ill and normal individuals. Continuous search for agents gives time advantages over spot epidemic investigations. Although finding the cause of infant diarrhea is the prime purpose, this investigation will have as a by-product information concerning the symbiotic relationships of certain of the newer viruses and their human hosts.

Proposed Course of the Project:

The clinical, epidemiological and bacteriological aspects of this program are carried out by Dr. Ralph Paffenbarger of the U.S.P.H.S. Robert A. Taft Environmental Health Center in Cincinnati. We propose to continue this project for at least one full year or until sufficient illness has occurred in the study population to justify the drawing of some definite conclusions.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. MIAIL-45

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$19,056	8,433	27,489	0.7	1.7	2.4

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	2	3

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. Ralph Paffenbarger, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-45
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None - new project.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-46
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Cellular antibodies.
PROJECT TITLE

7. Dr. John W. Hornibrook
PRINCIPAL INVESTIGATOR

8. Dr. Karl Habel and Nancy C. Gregg
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To study the serological reactions of specific animal cell types with antisera prepared against them.

Methods Employed:

Antisera have been prepared against several types of human cell grown in tissue culture. These antisera are being adsorbed by various tissues in an attempt to demonstrate cell-specific antibodies. Complement fixation, precipitin and cytopathogenic reactions are studied.

Major Findings:

Antibodies can be produced against HeLa and human skin cells grown in tissue culture. There are marked cross reactions of these antisera with antigens of all human cells. Preliminary findings after adsorption indicate the presence of antibody which appears more specific for tissues having the ability to multiply rapidly.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-46
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Cellular antibodies.
PROJECT TITLE

7. Dr. John W. Hornibrook
PRINCIPAL INVESTIGATOR

8. Dr. Karl Habel and Nancy C. Gregg
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To study the serological reactions of specific animal cell types with antisera prepared against them.

Methods Employed:

Antisera have been prepared against several types of human cell grown in tissue culture. These antisera are being adsorbed by various tissues in an attempt to demonstrate cell-specific antibodies. Complement fixation, precipitin and cytopathogenic reactions are studied.

Major Findings:

Antibodies can be produced against HeLa and human skin cells grown in tissue culture. There are marked cross reactions of these antisera with antigens of all human cells. Preliminary findings after adsorption indicate the presence of antibody which appears more specific for tissues having the ability to multiply rapidly.

Significance to the Program of the Institute:

There would be broad implications if an antigen could be located in cells which by serological means could be shown to be cell type specific.

Proposed Course of the Project:

This project is being expanded to include studies of animal tumor antigens and their antibodies.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-46

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57:	27,658	12,269	39,927	1.0	1.5	2.5

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	2	2	4

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-46
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

List of Publications not Related Entirely to this Project.

Snake Bites. John W. Hornibrook. Amer. J. of Nursing, 56(6): 754-755, June 1956.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-47
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Cultivation of mitochondria in vitro.
PROJECT TITLE

7. Dr. H. G. duBuy
PRINCIPAL INVESTIGATOR

8. Dr. M.L. Hesselbach, Dr. C.F.T. Mattern, J.L. Showacre, M.A. Steigner
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

Cultivation of mitochondria in vitro in order to (1) cultivate viruses on subcellular elements, (2) determine the pathogenic nature of certain types of mitochondria, e.g., by serology.

Methods Employed:

In order to maintain mitochondria suitable for cultivation, the methods used encompass those which determine mitochondrial intactness at subsequent stages of cultivation, as microscopic and subvital staining techniques, metabolic techniques, etc.

Major Findings:

Mitochondria have been studied in a number of media. More than one of the techniques mentioned must be applied to determine mitochondrial intactness.

Significance to the Program of the Institute:

Furthering of our understanding of the possible role of mitochondria in pathology.

Proposed Course of the Project:

Further substantiation of the theory already presented by additional data. (Phytopathology, 33:766, 1943)

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-47

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	7,313	3,275	10,588	0.8	0.3	1.1

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	1	2

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-47
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-48
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Investigations of the chemical composition of Coxsackie A-10 virus and its host cell elements.
PROJECT TITLE

7. Dr. Carl F. T. Mattern
PRINCIPAL INVESTIGATOR

8. Dr. H. G. duBuy
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To obtain further information on the mechanism and intracellular site of virus multiplication by means of chemical analysis of purified virus and isolated subcellular elements.

Methods Employed:

Coxsackie virus has purified by procedures developed in this laboratory. Subcellular fractions are isolated by means commonly employed in cell fractionation. A method of chemical analysis applicable to both virus and cell fractions has been adapted from previously described methods of analysis of viruses and tissue fractions.

Major Findings:

Coxsackie virus has been hydrolyzed to yield a nucleic acid fraction which has been hydrolyzed into purine bases and pyrimidine nucleotides. Paper chromatography has separated

these bases which have been tentatively identified as guanine, adenine, cytidylic acid and uridylic acid. This analysis indicates that this virus is of the ribose nucleoprotein type.

Significance to the Program of the Institute:

An understanding of the chemical composition and mechanism of replication may contribute to the therapy of virus diseases.

Proposed Course of the Project:

Continuation of the quantitative analysis of this virus and its host cell fractions.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-48

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$4,713	2,112	6,825	0.5	0.1	0.6

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	-	1

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. W. C. Alford, NIAMD
Dr. F. S. Brackett, NIAMD

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-48
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Purification and Crystallization of Coxsackie Virus. C. F. T. Mattern and H. G. duBuy. Science, 123:1037, June 8, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

The following two publications, appearing in 1956, were reports on work done more than 3 years ago when Dr. C. F. T. Mattern was assigned to another unit:

Continued observations on Histoplasmin Sensitivity and Calcified Pulmonary Lesions in a Rural Community. C. F. T. Mattern, J. A. Bell, C. W. Emmons, B. J. Olson, and I. P. Powell. Public Health Monograph #39, Aug. 1956. Published in the Proceedings of the Conference on Histoplasmosis held at Excelsior Springs, Mo., Nov. 18-20, 1952.

Experimental Silicosis: Changes in the Disease in the Monkey Produced by "Arterializing" the Lung. H. W. Scott, Jr., C. R. Hanlon, B. J. Olson, G. L. Fite, and C. F. T. Mattern. Surgical Forum 6:279, 1956.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-49
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the latent period of EMC virus.
PROJECT TITLE

7. Dr. Carl F. T. Mattern
PRINCIPAL INVESTIGATOR

8. M. A. Steigner
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To obtain information on the latent period or "eclipse phase" of virus multiplication.

Methods Employed:

Growth curves of EMC virus in mouse brain are obtained by harvesting animals at various intervals after infection. Brains are fractionated by various procedures to find that method giving maximum recovery of infectivity.

Major Findings:

Preliminary results indicate that between 8 and 20 hours, the growth rate is linear when plotted as log infectivity titer vs. time. Extrapolation of these lines to time 0 coincides with the total theoretical dose when 10^5 or 10^3 LD_{50}'s are inoculated, taking into account a 1/10 dilution of inoculum into brain (.03 ml in .3 ml). The departure from linearity beyond 20 hours

Major Findings (continued):

coincides with the death of many animals and may be explained by somewhat slower virus multiplication in surviving mice. The departure below 8 hours is attributed to the eclipse phase in which, by analogy to bacteriophage, the virus is presumed to be noninfectious. However, in several experiments much of the "eclipsed" EMC virus has been recovered as infectious virus by varying the techniques of tissue fractionation.

Significance to the Program of the Institute:

If this phenomenon can be thoroughly documented it will contribute to our understanding of virus replication.

Proposed Course of the Project:

Continuation.

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PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-49

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$4,713	2,112	6,825	0.6	0.1	0.7

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	-	1

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-49
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-50
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Particle Counting. II. Bacteria and Subcellular Particles
PROJECT TITLE

7. Dr. Carl F. T. Mattern
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

An instrument for counting particles by electronic gating has been under study here for nearly three years. It has been shown highly accurate for counting particles of more than 2.5 micron diameter. The developer of this instrument feels he now has modified the instrument to be able to enumerate and "size" particles of less than 1 μ diameter. It is hoped to evaluate this instrument as a tool in quantitative bacteriology and subcellular physiology.

Methods Employed:

A major portion of this study will be collaborative. Instrumental counts (discreet particles and clumps) will be made on samples which are being counted by a variety of other methods (plate, tube, chamber) in the course of routine investigations by other groups. Analysis of counts by these several methods should permit evaluation of this instrument's potential usefulness in the field.

Major Findings:

A complete report of studies to date is in press: J. Applied Physiology (Jan. 1957). The instrument was found to be a more accurate counting standard than the hemocytometer chamber which was found to have inherent systematic errors heretofore not recognized. The instrument also proved able to rapidly give information on the size distribution of particles.

Significance to the Program of the Institute:

A rapid, accurate method for counting small particles, including distinguishing discreet particles and clumps would be extremely valuable as a tool in quantitative bacteriology and subcellular physiology.

Proposed Course of the Project:

This phase of the project is to be started with the idea that within two or three years of part time effort it should be completed.

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(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-50

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$8,355	3,741	12,096	0.8	0.1	0.9

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	-	1

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. F. S. Brackett, NIAMD

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-50
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Determination of Number and Size of Particles by Electronic Gating. I. Blood Cells. C. F. T. Mattern, F. S. Brackett and B. J. Olson. In Press. J. Applied Physiology (Jan. 1957).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-51
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Investigations of interference with enzymatic functioning of mitochondria as a mechanism for causing neoplasia.
PROJECT TITLE

7. Dr. Marie L. Hesselbach
PRINCIPAL INVESTIGATOR

8. M. A. Steigner, Dr. H. G. duBuy, J. L. Showacre
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

(1) It has been reported in the literature that rat tumors have been caused by repeated injection of a certain dye. (2) It is known that certain other dyes affect the action of mitochondrial enzymes. Therefore, the present investigation was set up to: (1) Produce tumors by repeated dye injection; (2) Study the effect of the same dyes on the functioning of mitochondrial enzymes, in order to contribute to the understanding of the neoplastic process on a microbiological level.

Methods Employed:

Routine animal injection, weight charting, palpating and recording of changes in treated area. Efforts to find methods which make dye injections tolerable to animals. Biochemical studies of the effects of dyes by manometry.

Major Findings:

One dye being routinely injected has no known effect on the enzyme systems tested. The other (reported in the literature to produce tumors) changes the rates at which endogenous and exogenous substrates are utilized. The maximum dose at which these two dyes can be routinely injected is now known, as well as the distribution they assume in the animal. The dye which has been reported to produce tumors seems to cause more noticeable changes at the injection site than the other.

Significance to the Program of the Institute:

Both exogenous and endogenous "viruses" have been implicated as the cause of cancer. The present study is an effort to determine the relation of mitochondria to these "viruses".

Proposed Course of the Project:

Continued routine injection of the two dyes under study at present. Continued efforts to find a method of injection which will allow animals to tolerate repeated doses of a third dye. Continued biochemical study of the effects of the dyes. Biochemical studies of such tumors as are produced, as well as of other organs of the treated animals. Collaboration with a cytologist and a pathologist to obtain information on the morphology of the tumors. Effort to correlate this information with the biochemical information obtained. Serial transfer of the tumors, to prove that the change is heritable.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-51

12. BUDGET DATA:

	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$4,937	2,201	7,138	0.5	0.1	0.6

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	[illegible]	-	-

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. W. C. Alford, NIAMD
Mrs. Evelyn Peake, NIAMD
Mr. Charles Remsburg, NIAMD

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-51
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-52
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Cytopathogenic effect in single cells in tissue culture.
PROJECT TITLE

7. Jane L. Showacre
PRINCIPAL INVESTIGATOR

8. H. G. duBuy, C.F.T. Mattern, M.L. Hesselbach and M.A. Steigner
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To study the effects of virus infection on single cells grown in tissue culture.

Methods Employed:

Single tissue culture cells are studied microscopically in phase contrast and dark field, using subvital staining reactions to determine changes in dehydrogenase activity, and the localization of these changes on the cell. Cine and still photomicrographic records are made.

Major Findings:

A tissue culture chamber, suitable for continuous microscopic observation of living cells for varying time periods, has been adapted for use. A number of tissue culture cell types have been examined for suitability as tools for morphologic studies of virus infection. Preliminary studies on the behavior of living uninfected control tissue culture cells are underway. Cinemicrographic records have been obtained.

Significance to the Program of the Institute:

Initial cellular reactions to virus infection can be elucidated. A more detailed study of the cytopathogenic effect might lead to a differentiation of the action of different viruses.

Proposed Course of the Project:

A detailed comparison of microscopic changes in living uninfected and virus-infected tissue culture cells is underway.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-52

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$4,179	1,861	6,040	0.4	-	0.4

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	-	-

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-52
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-53
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Biology of mitochondria and its relation to endogenous and viral diseases.
PROJECT TITLE

7. Dr. H. G. duBuy
PRINCIPAL INVESTIGATOR

8. Dr. M.L. Hesselbach, Dr. C.F.T. Mattern, J.L. Showacre, M.A. Steigner
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

Biological definition of normal versus pathogenic mitochondria.

Methods Employed:

Methods encompass application of cytochemical, metabolic, genetic and serological techniques to whole cells and isolated subcellular elements.

Major Findings:

A number of mitochondrial types from normal and malignant tissues have been compared cytochemically and metabolically. A theory has been proposed to explain quantitatively different enzymic activities of mitochondria of various origin on the basis of differences in physico-chemical stability between mitochondrial types.

Significance to the Program of the Institute:

Furthering of our understanding of pathogenicity at a sub-cellular level.

Proposed Course of Project:

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-53

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$4,571	2,040	6,611	0.3	0.2	0.5

BUDGETED POSITIONS		
PROF	OTHER	TOTAL
-	-	-

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. W. C. Alford, NIAMD
Charles Remsburg, NIAMD

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-53
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Carbohydrate and carbohydrate metabolite utilization by enzyme systems of mouse brain and liver mitochondria. H. G. duBuy and M. L. Hesselbach. J. Histochem. and Cytochem. 4:363-376, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-54
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Basic Studies Section
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Characterization of mitochondria.
PROJECT TITLE

7. Jane L. Showacre
PRINCIPAL INVESTIGATOR

8. H.G. duBuy, C.F.T. Mattern, M.L. Hesselbach, M.A. Steigner
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

Objectives:

To discover the biochemical nature of the specificity of mitochondrial subvital staining with Janus green B.

Methods Employed:

The staining, decoloring and recoloring of mitochondrial elements, following the application of Janus green B, is studied with various microscopic techniques. Biochemical reactions are studied with the Warburg technique. This project has been expanded to include other dyes and has been extended. The information gained has established new reliable criteria for the definition of intact mitochondrial elements. It has supplied a useful tool for the demonstration of the presence and nature of certain dehydrogenases in various preparations resulting from cell fractionation.

Major Findings:

The mitochondrial staining reaction with Janus green B has been further analyzed. Janus green B and phenazine have been found to distinguish between glycolytic and Krebs cycle dehydrogenases under certain conditions.

Significance to Program of the Institute:

Establishment of a method to properly identify mitochondria and further elucidation of the nature of dehydrogenase systems.

Proposed Course of the Project:

Manuscript being prepared and project continued.

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October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-54

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$1,238	555	1,793	0.1	-	0.1

BUDGETED POSITIONS		
PROF	OTHER	TOTAL
1	-	-

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SMALL NO(S):

Mr. Charles Remsburg, NIAID
Dr. W. C. Alford, NIAID
Dr. D. L. Vivian, NCI

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-54
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-55
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3.
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Tuberculosis
PROJECT TITLE

7. Dr. B. J. Olson
PRINCIPAL INVESTIGATOR

8. Dr. G. W. Lones, Dr. B. Prescott, Miss Gladys Kauffmann
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION

A. Development of an Effective Killed Vaccine against Tuberculosis.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID-55
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3.
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Tuberculosis
PROJECT TITLE

7. Dr. B. J. Olson
PRINCIPAL INVESTIGATOR

8. Dr. G. W. Lones, Dr. B. Prescott, Miss Gladys Kauffmann
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION

A. Development of an Effective Killed Vaccine against Tuberculosis.

Objective:

The basic central project of the Unit is the development of an effective killed vaccine for tuberculosis. The effectiveness of U.V. killed vaccine was demonstrated in experimental animals in this Unit, and subsequently confirmed in other laboratory studies. This is a basic study essential to establish the antigenic component(s) prior to investigation of the relationship between antigenicity, immunological paralysis, and hypersensitivity.

Methods:

The methods currently employed are all inadequate. Therefore intensive efforts have been made to improve the methods of:

1. Determination of viable numbers of tubercle bacilli in the challenge dose.

2. Routes of infection to give consistent mortality curves.

3. Dosages of antigen in respect to quantity and time of administration.

4. Detection of presence or absence of viable organisms after irradiation.

Various strains of acid fast bacilli including virulent human strains were grown and harvested at five points on the growth curve. The following procedures of study were used: a) virulence test, using standardized doses in guinea pigs; b) viable counts on both the culture prior to harvest and on the centrifuged washed cell suspensions; c) settling rates as characteristic of particle size of suspension; d) optical densities; e) respiration determinations were intended to provide an overall measure of metabolic activity of these aerobic organisms as a function of the physiological age of the cells; respiratory activity of cell suspensions was determined in terms of uptake of oxygen in the Warburg apparatus at 37°C; f) catalase activity determinations were included as an additional measurement of a specific metabolic activity. Recently there has been an increased interest in this enzyme in the tubercle bacillus because of its relationship to isoniazid resistance. Catalase activity was assayed on similar suspensions in the same apparatus at 18°C. The oxygen liberated from hydrogen peroxide was measured; g) the tetrazolium reduction test has been proposed by others as a rapid measure of the viability of tubercle bacilli. The reduction test was performed by incubation of a cell suspension with 2,3,5-triphenyltetrazolium chloride followed by extraction of the resulting formazan with solvent and measurement in the spectrophotometer; h) for chemical study of these strains of tubercle bacilli during growth, analyses were made according to known procedures for carbohydrate, lipid

(phospholipid and wax), nitrogen, phosphorus, deoxyribonucleic acid and ribonucleic acid, purines and pyrimidine derivatives.

Major findings:

The various strains differ considerably in their activities and also in the effect of physiological age on these activities. In general, as would be expected, highest activities were found in cells from young cultures.

The carbohydrate, total lipids, deoxyribonucleic acid and total purine and pyrimidines increased to a maximum in about 12 days with all strains studied, and then decreased gradually with increasing age of the culture. The other components, nitrogen, phosphorus, ash, and ribonucleic acid remained essentially stable. Some variation was noted among the strains in content of each of the components.

Significance:

When correlated with the metabolic and cultural studies, the accumulated data should demonstrate the optimal conditions of growth to give maximum yields and to produce a satisfactory potential antigen.

Proposed course:

In view of the ultimate goal, the emphasis will now be laid on separation, purification and immunologic tests of fractions isolated at the indicated optimal growth period. The immunologic tests will include both immunization and sensitivity reactions.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 55
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	33,990	15,068	49,058	1.4	3.2	4.6

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	2	3	5

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH, INDICATE SERIAL NO(S):

NONE

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-55
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 56
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR SERVICE

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of Bacterial Agents in the Junior Village Population
PROJECT TITLE

7. Byron J. Olson
PRINCIPAL INVESTIGATOR

8. Dr. Elizabeth Verder

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION:

A. Studies of Bacterial Agents in Relation to Illness in the Junior

Village Population.

Objective:

The objective of this new project initiated on September 19, 1955 is to search for bacterial agents responsible for or associated with febrile illness in the Junior Village population. Particular emphasis will be placed on the relation, if any, between pathogenic bacteria and viral agents in both normal and ill children under observation.

Methods:

Initially, the occurrency and types of bacteria of the upper respiratory tract capable of causing deaths in mice injected both intracerebrally and intraperitoneally is being determined.

The pathogenic bacteria present in the G.I. tract will be subsequently studied.

Newer quantitative techniques will be used to determine populations and types of organisms in the upper respiratory tract and the lower G.I. tract.

Major findings:

In a series of over 800 mice injected in six consecutive weeks with material from the study group, the following mortalities of mice injected intracerebrally was obtained: 44%, 44%, 59%, 63%, 46% and 36%. The technique thus yields results of some consistent pattern.

Significance:

The simultaneous presence of pathogenic bacteria and pathogenic viruses in the same population of people and in the same individual poses one of the most perplexing problems in the etiology of disease. The bringing to bear the resources of the Epidemiological Section, the Virus Section and the Bacterial and Mycotic Disease Section on the same study population of patients should result in some light being shed on this perplexing problem.

Proposed Course:

Follow the leads as they develop in results obtained.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 56
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	13,046	5,794	18,840	0.4	1.5	1.9

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	- -	2	2

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

NONE

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-56
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-57
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Chemical Studies of the Tubercle Bacillus
PROJECT TITLE

7. Dr. George W. Lones
PRINCIPAL INVESTIGATOR

8. Dr. Byron J. Olson, Dr. Benjamin Prescott and Gladys Kauffmann
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION

A. Investigation of the Relationship between Metabolism and Isoniazid Resistance in the Tubercle Bacillus.

Objectives:

It is now recognized that strains of tubercle bacilli that have acquired marked resistance to isoniazid have altered virulence and de-

pressed or completely negative catalase activity. It is the objective of this project to examine further the metabolism of resistant strains and to compare them with sensitive strains with the expectation of extending our knowledge of the mechanism of resistance and sensitivity to isoniazid. Specifically, since catalase activity is dependent upon a ferriporphyrin-containing enzyme, the porphyrin relationship of sensitive and resistant cells is under study.

Methods Employed:

Catalase determinations are made in the Warburg respirometer at low temperatures. Peroxidase determinations depend upon the development of a colored substance from benzidine and hydrogen peroxide. Resistant and sensitive strains of tubercle bacilli are grown and harvested, preferably on porphyrin-free media. The organisms are then examined for porphyrins by chemical treatment and solvent extractions followed by solvent fractionations, and finally, quantitative measurements with a photofluorometer.

Major findings:

Isoniazid resistant, catalase negative strains of the tubercle bacillus have been carried for a year with about a dozen serial transfers on porphyrin-free media. These cells were found to contain porphyrin and are therefore capable of synthesizing this structure. This observation has recently been confirmed in a report by Middlebrook. The catalase-negative character of INH-resistant tubercle bacilli is therefore not due to their inability to form the porphyrin structure.

Significance:

The importance of an understanding of the mechanism by which a microorganism becomes resistant to a therapeutic agent is obvious. Added interest to this particular problem in Mycobacterium tuberculosis arises from the observation of others that isoniazid-resistant strains of the tubercle bacillus have a greatly reduced virulence, at least for the guinea pig. A better understanding of the altered metabolism of these strains may contribute to our knowledge of the factors influencing virulence and invasive ability.

Proposed course:

It is proposed that this problem be discontinued at this time, and be replaced by a study of the mechanism of tuberculin hypersensitivity to be initiated in the coming year.

B. Investigation of Metabolism of Nitrogen Compounds by Tubercle Bacilli.

Objectives:

It is the objective of this project to add to our knowledge of the nitro-

gen metabolism of this organism by the application of some of the techniques which have proved fruitful for this purpose in other bacteria. The role of the metabolism of nitrogen compounds in problems of virulence and resistance is inadequately understood.

Methods:

Warburg respirometer and chemical analytical methods are used with such modifications as are dictated by considerations for the virulence of the organisms studied. A Warburg bath has been modified to provide for automatic pasteurization of the reaction vessels at the end of an experiment.

Major findings:

It was previously found that of 22 amino acids studied, L-cysteine was the one most rapidly deaminated by resting cells of BCG. This reaction has been studied further. Acetone powders or cell-free extracts of BCG are more active than resting cells and these two types of preparation have been employed for the recent work. Although cysteine desulfhydrase activity is widely distributed in tissues and microorganisms, the mechanisms of the reaction is unknown. A number of workers have offered evidence that the reaction proceeds in two or more steps. A postulated intermediate in the reaction is thiol lactate. DL thiol lactic acid was synthesized and found not to be attacked by acetone-dried BCG. It therefore seems unlikely that this substance is an intermediate in the reaction.

Significance:

Our knowledge of the metabolism of the acid-fast bacteria has lagged far behind that of many other microorganisms. It is hoped that improved knowledge of the metabolism of these organisms may contribute to a solution of some of the problems they create.

Proposed course:

It is proposed to extend this study to other strains of the tubercle bacillus including virulent strains.

C. Isolation and study of Cerolytic Enzymes.

Objective:

It is the object of this project to obtain enzyme preparations capable of hydrolyzing the "wax" component of the tubercle bacillus. The literature contains a number of reports that such enzymes, prepared from microorganisms and wax eating insects, have antibacterial properties with respect to the tubercle bacillus, and that they are capable of favorably altering the course of infection with this organism in experimental animals.

Methods:

Classical enrichment techniques in liquid media containing waxes, particularly tubercle bacillus wax, have been followed by isolation attempts on agar containing wax emulsions with and without suitable indicators. Inocula for the enrichment cultures have included soil and the digestive tracts of larvae of Galleria mellonella, the wax moth.

Major findings:

A Streptomyces sp. previously isolated from the soil and capable of attacking beeswax has been examined further. Conditions of cultivation to produce maximum activity have been examined. Concentrates of the active component have been prepared and the ability to produce ether soluble acids from beeswax emulsions has been measured. Although this organism produces no visible alteration in purified tubercle bacillus wax emulsified in an agar medium, cell-free enzyme preparations possess about the same activity toward tubercle bacillus wax emulsions as toward beeswax emulsions. We have been able to detect no activity of these preparations on living tubercle bacilli. To date cells treated with the enzyme retain unaltered their staining reactions and viability.

Colonies of Galleria mellonella, the wax moth, have been maintained in the laboratory on beeswax. Attempts to isolate wax digesting microorganisms from the larvae of these insects have not been clearly successful. Presumptive activity of several isolates has not yet been confirmed.

Significance:

In addition to possible therapeutic activity of the "cerase" sought in this project, it is possible that such preparations might be useful research tools in the study of the chemical structure of the waxes of the tubercle bacillus and in the lysis of the tubercle bacillus to release intracellular enzymes for cell-free metabolic studies.

Proposed course:

Currently attempts are being made to extract lipolytic enzymes from Galleria larvae fed on wax. If active preparations are obtained, their properties will be studied with respect to the tubercle bacillus.

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(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 57
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	18,809	8,353	27,162	1.2	1.2	2.4

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	1	1	2

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

NONE

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. _____
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-58
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR SERVICE

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Synthesis and Tests of Potential Chemotherapeutic Agents against Mycobacterium Tuberculosis
PROJECT TITLE

7. Dr. Benjamin Prescott
PRINCIPAL INVESTIGATOR

8. Mr. Junius Webb
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION

Synthesis and Tests of Potential Chemotherapeutic Agents against Mycobacterium tuberculosis.

Objectives:

To isolate effective, nontoxic compounds for use in therapy of human tuberculosis.

Methods:

Derivatives of benzothiazole were synthesized by condensing various aromatic aldehydes with O-aminothiophenol in 95% ethyl alcohol. The final recrystallized products were analyzed for carbon, hydrogen, nitrogen, and tested for melting points. Toxicity studies were run in DBA mice. Activity was assayed by measuring the bacteriostatic effect in vitro on Mycobacterium tuberculosis.

Major findings:

Of 51 crystalline products synthesized, none was as toxic as isoniazid, the currently used tuberculostatic agent. Three, 2-(o-methoxyphenyl) benzothiazole, 2-(o,m-methoxyphenyl) benzothiazole, 2-(o-ethoxyphenyl) benzothiazole, with only 1/20 the toxicity, showed activity in in vitro tuberculostatic tests as great as that of isoniazid.

Significance:

Because of high tolerance and effectiveness, these three benzothiazoles may be useful in the chemotherapy of human tuberculosis.

Proposed course:

Tests for usefulness of these compounds are to be run to determine their effectiveness in experimental tuberculosis in laboratory animals.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 58
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	7915	3517	11432	0.2	1.0	1.2

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	--	1	1

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

NONE

(Use reverse and additional pages, if necessary)

Form No. ORP-1
Calendar Year 1956

SERIAL NO. NIAID - 58

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

Part C: Honors, Awards & Publications

15. NIAID - 58
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

17. LIST HONORS AND AWARDS TO BE CONSIDERED RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. ORP-1
October 1956
(Attachment I)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 59
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR SERVICE

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Chemotherapy of Tuberculosis: A - Detoxication Studies
PROJECT TITLE

7. Dr. Benjamin Prescott
PRINCIPAL INVESTIGATOR

8. Miss Gladys Kauffmann
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION (SEE INSTRUCTIONS):

A1. Studies on the toxicity and the detoxication of cycloserine, a new antibiotic used in the treatment of human tuberculosis.

Methods Employed:

Simultaneous administration orally of cycloserine and various potentially detoxifying chemicals in DBA mice in attempts to reduce the "circling" activity which is always observed with doses of 25 mg per mouse or more.

Major Findings :

Various amino acids, vitamins, metabolites, other antitubercular agents, some alkaloids have been tested orally in both simultaneous and staggered treatments with cycloserine. A few compounds (reserpine, isoniazid, and alpha ketoglutaric acid particularly) show promise.

Significance:

Greater therapeutic effectiveness against infections with tubercle bacilli may be achieved if dosages of cycloserine may be increased, without or with reduced toxic manifestations.

Proposed Course:

The study is being continued on the few compounds that show promise.

A2. Increase in the Tolerated Dose of Isoniazid in DBA Mice by the use of Various chemicals.

Objectives:

To find further means of reducing the toxicity of this widely used tuberculostatic agent.

Method:

The effect of various chemicals on the activity of isoniazid (INH) was measured by (a) the effect on survival time of normal mice following simultaneous oral administration of a given chemical with a lethal dose of INH (both single and multiple administrations); (b) the effect on blood plasma levels of INH, as measured by the "pyridyl" method; and (c) the effect on INH bacteriostatic action in vitro against tubercle bacilli.

Major Findings:

With amino acids: On single administration of a 10 mg dose of INH per 20 gram mouse, four amino acids permitted 50 to 62% survival of DBA mice, namely, l-cysteine-HCL, l-glutamic acid-HCL, acetyl-dl-methionine, and hydroxy-l-proline; 15 other amino acids tested showed little or no activity. With an 8-mg dose of INH, the same amount of l-cysteine-HCL permitted 100% survival. On repeated administration at 72-hour intervals, this

dosage combination was tolerated for 15 doses by 60% of the mice.

With alpha-keto acids: Of six alpha-keto acids, pyruvic and alpha-ketoglutaric acid (neutralized) were effective in doses of 30 and 10 mg respectively in permitting survival of mice given 10 mg INH at the same time, in both single and repeated doses.

With certain vitamins: Of 16 vitamins similarly tested, three showed some detoxifying action, namely, nicotinic acid, p-aminobenzoic acid, and pyridoxal-HCl. The last named (neutralized) gave optimal results in combination with 6 to 10 mg INH in either single or repeated oral administration.

With cycloserine: Mice survived an 8-mg oral dose of INH when combined with 75 mg cycloserine, or a 10 mg dose with 100 mg cycloserine. Seventy-three percent of the mice survived 20 semi-weekly administrations of the latter mixture.

With glycerine as solvent: With a lethal 6-mg oral dose of INH, solution in 35% glycerine (in water) permitted 95% survival of a group of 118 DBA mice; and with 8 mg INH, dissolved in 50% glycerine, 86% of 146 mice survived. However, on repeated administrations, optimum results with no toxic manifestations were found in mice given 4 mg (sublethal) dose in 35% glyceride solution.

With propylene glycol as solvent: Studies initiated with propylene glycol as solvent for INH have thus far shown that a single oral administration of 10 mg INH in 75% propylene glycol in water was tolerated by 90% of 149 DBA mice.

Major Findings: (b) and (c)

Blood plasma levels were measured following administration of INH with each of the effective chemicals mentioned above. As anticipated, higher INH levels were obtained than with the tolerated dose (4 mg) of INH alone, due to the possibility of using larger amounts of INH.

No interference by the detoxifying agents tested was observed in the in vitro bacteriostatic action of INH on the strains of Mycobacterium tuberculosis tested.

A3. Increase in the Tolerated Dose of Streptomycin in White Mice by the use of Various Chemicals.

Objectives:

To find further means of reducing the toxicity of this widely used tuberculostatic agent.

Methods:

The effect of various chemicals on the activity of streptomycin was measured by (a) the effect on survival time of normal mice following simultaneous oral administration of a given chemical with a lethal dose of streptomycin (both single and multiple administrations); (b) the effect on blood plasma levels of streptomycin; and (c) the effect on streptomycin bacteriostatic action in vitro against tubercle bacilli.

Major Findings:

A 10 mg/20 gram mouse lethal dose of streptomycin is tolerated by 90% or more of the mice when administered by subcutaneous route simultaneously with either (1) a mixture of 50 mg glycine and 50 mg sodium glucuronate; (2) 75 mg alpha-ketoglutaric acid; (3) in 25% glycerine; or (4) in 50% propylene glycol. Thus far only glycerine solutions of streptomycin have been tested to determine the effect of the solvent of the in vitro bacteriostatic action of the antibiotic, with the result that the solvent enhances the inhibition of growth of tubercle bacilli.

Significance:

As with isoniazid above, the widespread use of the antibiotic streptomycin in the treatment of human tuberculosis is limited by the toxicity. Hence means of increasing the tolerance should permit more effective therapy.

Proposed Course:

This study will be extended to include bacteriostatic tests similar to those above.

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(Attachment I)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 59
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	13,763	6,114	19,877	0.7	1.3	2.0

	PROF	OTHER	TOTAL
FY'57	1	2	3

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSISTANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH, INDICATE SERIAL NO(S):

NONE

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. _____

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Felton, L.D., Prescott, B., Kauffmann, G. and Ottinger, B. Pneumococcal antigenic polysaccharide substances from human tissues. J. Immunol., 76, 69, 1956.

Prescott, B., Kauffmann, G. and James, W. D. A Means of Reducing Hydrazine Toxicity in Mice. A.M.A. Arch. Indus. Health, 13, 24, 1956.

Katz, S., Gimble, A., McCormick, G., and Prescott, B. Glycine and Sodium Glucuronate in the Management of Convulsions due to Hydrazides. Annal. Int. Med., 44, 576, 1956.

Webb, J. M. A sensitive method for the determination of ribonucleic acid in tissues and microorganisms. J. Biol. Chem., 221, 635, 1956.

Prescott, B., Kauffmann, G. and James. W. D. Increase in the Tolerated Dose of Isoniazid in Mice by the Use of Cycloserine. Proc. Soc. Exp. Biol. Med. (In press).

Prescott, B., Kauffmann, G. and James. W. D. The effect of Glycerine on the Toxicity of Isoniazid in Mice. Proc. Soc. Exp. Biol. Med. (In press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. CRP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIH - 30
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR SERVICE 3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE 5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Purine and Pyrimidine Derivatives in Tubercle Bacilli
PROJECT TITLE 1. Procedures 2. Applications

7. Miss Gladys Kauffmann
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT PRESENTS, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT DUPLICATION OF RECOMMENDED FACILITIES OR FUNDS), IDENTIFY SUCH PROJECT: (BY SERIAL NO. (S) IF OTHER THAN).

None

10. PROJECT DESCRIPTION

Studies on Purine and Pyrimidine Derivatives in Tubercle Bacilli.

Objective:

To modify the silver precipitation method of separation of purines and pyrimidine nucleotides for use with tubercle bacilli and fractions thereof; to ascertain effect of components other than nucleic acids on the assay for purine and pyrimidine nucleosides and nucleotides; to assay the amount of these components in desoxyribonucleic acid isolated from tubercle bacilli; and to study the relative amounts in various strains of tubercle bacilli at different stages of growth.

Methods:

Separation of the individual purines from the pyrimidine nucleotides in the hydrolyzed tubercle bacilli by an adaptation of a silver method. Amounts are assayed by optical density measurements at predetermined wave lengths from 240 to 280 mu to indicate the amount of the individual purines, and similarly from 260 to 295 mu for the pyrimidine derivatives.

Major findings:

Procedure has been modified to assay the purine and pyrimidine derivatives in dried tubercle bacilli. The approximate ratio of total purine to pyrimidine nucleoside was found to be 1:2. In the various strains studied, the highest levels of both constituents were found in those extracted from vole bacillus and from the human strain H37Rv tubercle bacillus.

Significance:

The ultimate goal of the project is to ascertain the relationship of these constituents to infectivity, to hypersensitivity, and to immunity.

Proposed Course:

Study of the effect of other constituents of the tubercle bacillus will be continued. When assay on the various strains is completed to establish relationship to cultural characteristics, these components are to be tested for their effect on tuberculin allergy or immunity, as well as the desoxyribonucleic acid itself isolated in the native state.

B. Chemical Studies of the Vole Bacillus.

Objective:

In view of the extensive use in Britain of this microorganism as an active immunizing vaccine against tuberculosis, the objective of the present project is to isolate and purify the actively antigenic components(s) from this bacillus in view of obtaining a more nearly ideal antigenic agent for human use than the current vaccine BCG.

Methods:

Fractions were isolated from a strain of this bacillus by procedures adapted in this laboratory, and include polysaccharides, phospholipids, waxes, proteins, and nucleoproteins. These were analyzed for nitrogen, phosphorus, and in the case of the polysaccharides for reducing sugars after hydrolysis and for optical rotation. Attempts to identify constituent sugar molecules by paper chromatography and by infrared analysis were made on these polysaccharides in comparison with the corresponding polysaccharides from BCG. Specific and nonspecific precipitin tests were run with homologous antiserum and with BCG antiserum.

Major findings:

Analytical data showed considerable similarity of the vole fractions and the corresponding fractions from BCG. In the serological tests significant cross reactions were observed with the vole polysaccharide and protein fractions and BCG antisera, and conversely between BCG fractions and vole bacillus antisera.

Significance:

From the results so far obtained the fractions isolated from the vole bacillus show at least as much promise as those from BCG for prophylactic use against Mycobacterium tuberculosis.

Proposed Course:

Future emphasis will be placed on the effect of these fractions in tests for tuberculin allergy and for active immunity against experimental tuberculosis in laboratory animals.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 60
SERIAL NUMBER

12. BUDGET DATA:

	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	2,550	1,120	3,670	0.3	- -	0.3

	BUDGETED POSITIONS		
	PROF	OTHER	TOTAL
FY'57	- -	- -	- -

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

NONE

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. MI/ ID-60
SPECIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part I. Project Description Sheet 1. NIAID - 61
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. The effect of Animal Virus Reproduction on Host Cell Metabolism
PROJECT TITLE

7. Dr. Hilton B. Levy
PRINCIPAL INVESTIGATOR

8. NONE
OTHER INVESTIGATORS

9. IF THIS PROJECT RECEIVES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT DUPLICATION OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF IT IS KNOWN).

NONE

10. PROJECT DESCRIPTION:

Objectives: To gain information relative to the relationships that exist between the diseased cell and the virus reproducing therein. It is anticipated that this program will elucidate some aspects of the mechanism of virus reproduction. In addition, better understanding of the nature of the disease response may be forthcoming from a determination of which of the specific paths of cell metabolism are altered by the disease process. Knowledge of either or both of these phases of the problem (virus reproduction and altered cell metabolism) might be useful in the development of chemotherapeutic agents to inhibit virus growth and to aid the host.

Methods. The experimental approach to this problem involves an attempt to infect uniformly a population of host cells with the virus under study, and then to examine the virus-host system by suitable laboratory techniques for several metabolic activities. As mentioned in the report of Dec. 1955, (NMI-70,E., 1955) the desirability of working with a uniformly infected cell population has caused us to shift from ascites tumor cells in vivo as the host tissue, to tissue culture, but the latter is available only in rather limited quantity. We have spent some time this past year investigating techniques that will enable us to use smaller amounts of tissue than were previously thought to be required. (For details see section on major findings.) As in the past, physicochemical methods such as manometry, radioisotope techniques and spectrophotometry will play an important role in our experimental procedure, but an effort will be made to give some emphasis to the more biological and medical aspects.

Major Findings:

A. Polio Viruses. The increased glycolysis induced in monkey kidney tissue culture by polio virus infection has been found under anaerobic as well as aerobic conditions, which finding may have some relation to the mechanism of the effect. Other substances such as merthiolate and dyphtheria toxin which cause cell destruction have been compared with polio virus and found to have some features in common with but other features that are different from the virus.

The inhibition of uptake of radioactive glycine has been found to be maximal in the lipid fraction and minimal in the nucleic acid fraction.

In experiments to compare the effects of types I, II and III polio virus, we inadvertently made the observation that some control batches of monkey kidney tissue culture behaved metabolically in a manner similar to that which we had found for polio virus-infected cells. In each case where we found such results, the cells came down with cytopathogenic effects, and came off glass within 2-3 days. Limited attempts to find a transmissable agent in these cells have not been sucessful.

B. Adenovirus and Coxackie Viruses. With Dr. Rowe we have started a program to study the metabolic activities of the Hela-cell adenovirus system as well as that of the Hela cell-Coxackie virus system. Two strains of the latter have been used, one being highly oncolytic to solid Hela tumors in rats and the other much less so. As with the polio virus-monkey kidney system, we measured changes in cellular metabolism that were reflected in the medium by determining the lactic acid produced, and changes that were reflected in the cells by measuring uptake of radioisotope. Adenovirus produces markedly different visible effects on the cells, and very different metabolic effects were found also. With polio virus a decrease in the rate of glycine uptake was found within an hour after infection, while with the adenovirus a stimulation of glycine uptake was noted within two hours after infection which stimulation lasted for 96 hours. Both early periods of time are rather short in comparison with the latent period ascribed to the viruses.

With the adenovirus a comparable protracted stimulation in P^{32} uptake was noted. Preliminary experiments with polio virus suggest that this stimulation of P^{32} uptake is not found. The two Coxackie virus strains appear to be alike in that both induce a moderate inhibition of glycine uptake and little effect on P^{32} uptake.

Adenovirus infection of Hela cells, like polio virus in monkey kidney tissue culture, leads to a stimulation of lactic acid production but the stimulation by the former does not appear until about 10 hours after infection.

Significance: The intensive investigations of viruses conducted at N.M.I. are devoted largely to epidemiological, clinical or immunological aspects of viral diseases. The program described here should contribute somewhat toward rounding out the biochemical and metabolic phases of the research. In addition, it is to be hoped that this program will be of use to, and will draw on the knowledge gained in the more clinically directed programs. It is also hoped that a biochemical approach to the problem of latency in animal viruses and to the question of the relationships between viruses and cancer may be developed.

Proposed Course:

(1) An investigation of possible enzymatic and/or permeability changes in the infected cells to seek and explanation of the rise in glycolysis.

(2) A study of the effects of the other polio types, as well as other viruses, to determine whether the above mentioned findings are universal or specific.

(3) An investigation of the more detailed effects of adenovirus on nucleic acid metabolism. The nucleic acids being essentially polymerized nucleotides with carbon-containing bases and phosphate groupings lend themselves well to a detailed analysis of internal turnover. This would be done through the use of double labelling with P^{32} and C^{14}, followed by chemical and/or enzymatic hydrolysis of the separated nucleic acids to their constituent nucleotides, separation of the nucleotides by paper electrophoresis and determination of the specific radioactivity of the p^{32} and C^{14}. This should enable us to get as close, biochemically speaking, to the reproductive mechanisms of animal viruses as any approach currently available.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-61
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,315	$6,841	$22,156	0.9	1.1	2.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. Samuel Baron, DBS

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications: 15. NIAID - 61
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS BY THIS PROJECT DURING CALENDAR YEAR 1956:

Levy, Milton S. & Goldbaker, LeRoy E., Effects of Animal Viruses on Host Cell Metabolism I - Effects of E.M.C. Virus on Purine Acid & Pyrimidine fractions of Ehrlich Ascites Cells. J. Inf. Diseases, 98, 271-276, 1956.

Levy, Milton S. and Baron, Samuel, Some Metabolic effects of Poliomyelitis Virus in Tissue Cultures, Nature (In Press)

Levy, Milton S. and Baron, Samuel, Effects of Animal Viruses on Host Cell Metabolism II - Effect of type III poliomyelitis virus on Glycolysis and uptake of Glycine 2-C14 by monkey kidney tissue cultures. J. Inf. Diseases (In Press).

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 62
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Nucleoproteins and Nucleic Acids as Antigens
PROJECT TITLE

7. Dr. Hilton B. Levy
PRINCIPAL INVESTIGATOR

8. Dr. Benjamin Prescott
OTHER INVESTIGATOR

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION

Objectives: In view of the biochemical specificity and genetically fundamental character of the nucleic acids, particularly desoxyribosenucleic acid, together with their large molecular size, it is planned to investigate their possible activity as antigens. The production of different types of antibodies will be studied, as well as possible immunizing action. Genetic similarities among different strains of organisms will be investigated by means of cross-reactivities of sera with non-homologous nucleic acids. The purine and pyrimidine composition of the ribosenucleic acids and desoxyribosenucleic acids isolated from the different strains of bacteria will be determined and the results analyzed in light of possible immunological similarities or relationships.

The program is restricted, at least for the present, to various strains of acid-fast bacteria.

Methods: We have developed new methods for the preparation of highly polymerized nucleic acids and nucleoproteins from a variety of bacteria. For this study we have prepared nucleic acids from the BCG, the avian and the Vole varieties of the tubercle bacillus. When suitable safety precautions have been worked out, preparation of nucleic acids from the human tubercle bacillus will be undertaken. Standard techniques will then be used for testing for antigenicity of the nucleic acids and for the development of immunity in the test animals. Paper chromatography will be used for the chemical analysis of the nucleic acids.

Major Findings: This program has been almost completely inactive this past year. We did find that these preparations are capable of producing C. F. antibodies in mice by I.P. or I.V. routes. Traces of amino acids have been found in the hydrolyzates of the nucleic acids from mycobacterium. Whether or not these small quantities of amino acids indicate the presence of sufficient protein to account for the antigenic character of the nucleic acid preparations remains to be seen.

Significance: The fact that antigenic activity has been demonstrated in nucleic acid preparations (Project No. NI-70,F., 1955) immediately raises the question as to the possible role of such antigens in immunological specificity. In view of the history of the poor relationship between production of circulating antibody and immunity in tuberculosis, it is not likely that this antigen will be of value in the prophylasis of tuberculosis, but this possibility must be investigated. Whether nucleic acid antigens might prove useful in differential diagnosis of bacterial diseases could be investigated.

Proposed Course: In cooperation with Dr. Olson and Dr. Prescott, we plan to determine whether or not these antigens produce any degree of immunity in guinea pigs. The effect of nucleic acid hydrolyzing enzymes will be studied to determine whether or not the nucleic acids themselves are the active antigenic material.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-62
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$10,514	$4,699	$15,213	0.1	0.1	0.2

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57				

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGIC STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID - 62
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID-63
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Iron-binding Proteins as Antigens and Iron Metabolism in Allergic and Hyper-sensitive States and Infectious Diseases
PROJECT TITLE

7. Arthur L. Schade, Ph.D.
PRINCIPAL INVESTIGATOR

8. Robert C. Woodworth, Ph.D.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION

A. The specifically iron-binding proteins Conalbumin and Siderophilin, as exceptional antigens for study of antibody production and antigen-antibody reactions.

Objectives:

To investigate the immunological activity of these readily radioactively-tagged proteins in suitably prepared animals and, by appropriate techniques, to correlate this activity with observable physio-

logical and histological reactions of the host.

Methods:

Procedures for preparing the proteins and tagging them with Fe^{59} are those of the investigator. Radioactivity counting and radioautographic techniques, immunologic procedures for preparing immune animals and obtaining immune sera, antigen-antibody analyses, as well as post-mortem evaluations of immunological responses are performed.

Major findings:

Conalbumin from chicken egg white and human siderophilin are excellent antigens for the production of precipitating antibodies by the rabbit. Iron-saturated tagged conalbumin is easily followed as an antigen in immunologic studies from which it has been shown that the iron of such a complex can be removed by the rabbit without destruction of the protein moiety. Procedures have been developed for estimating the rate of protein and tagged protein removal from the blood stream. For short term experiments, within one hour, the radioactivity measurements serve as reliable indexes of the antigen disposition. The lung, weight for weight, has been shown to be the organ of greatest significance for antigen-antibody removal from the circulating blood.

Significance:

The use of readily tagged antigens, whose integrity is not affected by the procedure of tagging, provides means for intimate study of antibody-antigen reactions, antigen localization in body tissues, and association of such localization with the organs involved in antibody production, asthmatic conditions, anaphylactic shock, allergic responses to infection, etc.

Proposed course:

We propose to investigate the conditions which affect the localization of antigen-antibody complexes in the organs of the body, particularly the lung, and associate such localization with physiological and possible anatomical changes in lung tissue consequent upon induced antigen-antibody interaction. A study of changes in formed blood components resulting from such interaction will be made in collaboration with Dr. James Stengle of NCI.

B. Methods for the determination of the total iron-binding capacity of blood plasma, the amount of bound iron in plasma, and the amounts of free and bound iron in microbiological media.

Objectives:

To perfect methods for the determination of iron, both free and bound, in body fluids and in media of microbiological importance to aid in the study of the iron metabolism of the host, as well as of the infectious agents.

Methods:

Basic methods described in Methods in Medical Research, 1956, to be published.

Major findings:

Iron analyses can be quickly and accurately performed on as little as 0.1 ml of plasma with a reproducibility of ± 5%. No protein removal is required either in the estimation of siderophilin-bound iron or of the total binding capacity of the plasma sample.

Significance:

The availability of a simple, reliable method for the total iron-binding capacity of blood is of value for the study of all disease states in which iron metabolism is disturbed by allergic and infectious disease.

Proposed course:

We propose to extend our methodological investigations to permit ready iron estimation in plasmas of dogs and rats. Special problems have arisen in the use of those plasmas both by us for some projected iron metabolism studies and by the U. S. Naval School of Aviation Medicine at Pensacola, Florida, who have sought our aid in this matter through their biochemist J. K. Colehour.

C. The Chemistry of the Iron-siderophilin Complex.

Objectives:

The aims of this project are to investigate the chemistry of the reactive groups in the siderophilin molecule responsible for the binding of iron and to determine the physical and chemical means by which the iron can be made labile under physiological conditions obtaining in pathologic and normal hosts.

Methods:

Methods include the employment of radioactive tracer techniques, potentiometric titrations, spectral analyses, polarimetry, quantitative elemental analyses, ion-exchange chromatography, and electrophoresis.

Major findings:

Much has already been established by the investigator and others of the gross in vitro chemistry of the iron-siderophilin combination. The in vivo conditions for iron release from this chelated complex are poorly understood.

logical and histological reactions of the host.

Methods:

Procedures for preparing the proteins and tagging them with Fe^{59} are those of the investigator. Radioactivity counting and radioautographic techniques, immunologic procedures for preparing immune animals and obtaining immune sera, antigen-antibody analyses, as well as post-mortem evaluations of immunological responses are performed.

Major findings:

Conalbumin from chicken egg white and human siderophilin are excellent antigens for the production of precipitating antibodies by the rabbit. Iron-saturated tagged conalbumin is easily followed as an antigen in immunologic studies from which it has been shown that the iron of such a complex can be removed by the rabbit without destruction of the protein moiety. Procedures have been developed for estimating the rate of protein and tagged protein removal from the blood stream. For short term experiments, within one hour, the radioactivity measurements serve as reliable indexes of the antigen disposition. The lung, weight for weight, has been shown to be the organ of greatest significance for antigen-antibody removal from the circulating blood.

Significance:

The use of readily tagged antigens, whose integrity is not affected by the procedure of tagging, provides means for intimate study of antibody-antigen reactions, antigen localization in body tissues, and association of such localization with the organs involved in antibody production, asthmatic conditions, anaphylactic shock, allergic responses to infection, etc.

Proposed course:

We propose to investigate the conditions which affect the localization of antigen-antibody complexes in the organs of the body, particularly the lung, and associate such localization with physiological and possible anatomical changes in lung tissue consequent upon induced antigen-antibody interaction. A study of changes in formed blood components resulting from such interaction will be made in collaboration with Dr. James Stengle of NCI.

B. Methods for the determination of the total iron-binding capacity of blood plasma, the amount of bound iron in plasma, and the amounts of free and bound iron in microbiological media.

Objectives:

To perfect methods for the determination of iron, both free and bound, in body fluids and in media of microbiological importance to aid in the study of the iron metabolism of the host, as well as of the infectious agents.

Methods:

Basic methods described in Methods in Medical Research, 1956, to be published.

Major findings:

Iron analyses can be quickly and accurately performed on as little as 0.1 ml of plasma with a reproducibility of ± 5%. No protein removal is required either in the estimation of siderophilin-bound iron or of the total binding capacity of the plasma sample.

Significance:

The availability of a simple, reliable method for the total iron-binding capacity of blood is of value for the study of all disease states in which iron metabolism is disturbed by allergic and infectious disease.

Proposed course:

We propose to extend our methodological investigations to permit ready iron estimation in plasmas of dogs and rats. Special problems have arisen in the use of those plasmas both by us for some projected iron metabolism studies and by the U. S. Naval School of Aviation Medicine at Pensacola, Florida, who have sought our aid in this matter through their biochemist J. K. Colehour.

C. The Chemistry of the Iron-siderophilin Complex.

Objectives:

The aims of this project are to investigate the chemistry of the reactive groups in the siderophilin molecule responsible for the binding of iron and to determine the physical and chemical means by which the iron can be made labile under physiological conditions obtaining in pathologic and normal hosts.

Methods:

Methods include the employment of radioactive tracer techniques, potentiometric titrations, spectral analyses, polarimetry, quantitative elemental analyses, ion-exchange chromatography, and electrophoresis.

Major findings:

Much has already been established by the investigator and others of the gross *in vitro* chemistry of the iron-siderophilin combination. The *in vivo* conditions for iron release from this chelated complex are poorly understood.

Progress can be expected when the more intimate chemistry of the complex itself is known.

Significance:

The shift in bound serum iron levels as a result of allergic reactions and infectious diseases is recognized. The increased lability of serum iron is conditioned by the chemistry of the protein-iron complex and the physiologic state of the host.

Proposed course:

We propose to employ purified and crystalline siderophilin from human blood plasma in the study of (1) the stoichiometry of the iron-siderophilin carbonate complex, (2) the location and nature of the siderophilin molecule's iron-binding sites, and (3) the stereochemical requirements of the siderophilin molecule for binding and unbinding of iron.

D. Serum Iron and the Total Iron-binding Capacity of Blood.

Objectives:

The aims of the project are threefold: to investigate the contribution of blood plasma siderophilin to the natural resistance of the host; to relate the fall in the level of serum bound iron as a result of infection and allergic reaction to activity of the reticulo endothelial system; and to evaluate the significance of the effect of nicotinic acid administration on the serum iron and bilirubin concentration increase in the blood.

Methods:

The usual immunological, biochemical, radioactivity and bacteriological procedures are employed. In addition, methods peculiar to siderophilin affinities for iron serve.

Major findings:

Investigations have shown that pathogenic bacteria, as well as saprophytic forms in a generally satisfactory growth medium, can be divided into three groups on the basis of their growth reaction to the addition of siderophilin to the medium. They are either completely, partially, or not at all inhibited by the siderophilin addition. Both the complete and partial inhibition is overcome by addition of an amount of iron in excess of the binding capacity of the added specific iron-binding protein. Human and rabbit blood sera which possess unsaturated iron-binding capacity occasion similar effects in addition to other inhibitory properties against certain bacterial forms.

Administration to humans of nicotinic acid by mouth or by the intravenous route leads to a 100% or more increase in the serum iron and bili-

rubin levels within one half to one hour which levels return to normal values in an additional one or two hours time. Such a rapid response in both bilirubin and serum iron invite study as to mechanism of their increase.

Significance:

The serum or plasma iron levels, as well as the concentration of siderophilin in the blood, are valuable indications of infectious disease states. The serum iron picture reflects the iron metabolism of the host and provides a useful tool for investigation of anemic conditions generally associated with infections. Further knowledge of the metabolism of iron, particularly by the leucocytes and the reticulo endothelial system as reflected by the serum iron levels is probably of significant value for the mobilization and activity of these agents in hypersensitivity reactions and immunological defense mechanisms of the host.

Proposed course:

We propose to add to our studies of the iron requirements of pathogenic and saprophytic bacteria as affected by siderophilin and to continue to be of assistance in clinical studies by L.C.I. of host iron metabolism in cases of infectious diseases; accumulation of iron from the serum by the reticulo endothelium as an immunological response will be investigated; studies on nicotinic acid administration and its effect on serum iron and bilirubin metabolism in humans and dogs will be undertaken with Dr. James Stengle of N.C.I.

Form No. [illegible]-1
October 1956
(Attachment 1)

PUBLIC HEALTH SERVICE—NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part 3: Budget Data

11. NIAID-63
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REINVESTMENT	TOTAL	PROF	OTHER	TOTAL
FY 1957	$28,691	$12,804	$41,495	2.0	2.6	4.6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY 1957	2	3	5	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGICS STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. James Stengle, NCI

J. K. Colchour, U. S. Naval School of Aviation Medicine, Pensacola, Fla.

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. MIAID-63
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Schade, Arthur L. Bound Iron and Unsaturated Iron-binding Capacity of Plasma. Accepted for publication in "Methods in Medical Research, 1956".

Burk, Dean and Schade, Arthur L. Respiratory Impairment in Cancer Cells. Science, 124, No. 3215, pp. 270-272, Aug. 10, 1956.

Stengle, J. M. and Schade, A. L. Diurnal-Nocturnal Variations of Certain Blood Constituents in Normal Human Subjects: Plasma Iron, Siderophilin, Bilirubin, Copper, Total Serum Protein and Albumin, Haemoglobin and Haematocrit. Accepted for publication, to appear in Jan. 1957 issue of British Journal of Haematology.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-64
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial and Mycotic Diseases
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Streptococcal Disease
PROJECT TITLE

7. Dr. Roger M. Cole
PRINCIPAL INVESTIGATOR

8. Edythe J. Rose
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

The identification and role of streptococci in the spectrum of human upper respiratory disease.

Objectives:

To determine the role of streptococci in upper respiratory disease, by isolating and identifying serologically the organisms, and relating their occurrence to the presence of disease and of other infectious agents.

Methods Employed:

Streptococci isolated are obtained in pure culture by plating and picking colonies to broth; acid extracts of the packed cells from broth cultures, after neutralization, are used as the antigens in the usual capillary-tube precipitation tests for identification of streptococcal groups and of types within Group A. Specific antisera for identification are

Methods Employed (continued):

supplied by the Streptococcus Laboratory, CDC, and some antisera are made in our laboratory. Pertinent hemolytic and biofermentative reactions, as well as growth characteristics, are also used in identification at times. Strains to be added to the collection are grown on blood-agar slants, suspended in sterile skim milk and lyophilized. A record of each strain and its characteristics is kept on an annotated keysorter punch card for ready reference.

Major Findings:

In 1956 (through 11/14/56), the Rheumatic Fever and Streptococcal Diseases Unit attempted identification of 499 cultures. The majority were received from the Influenza Vaccine Study (306) and from the Junior Village Study (40) -- 346 cultures in all. Both of these are studies of upper respiratory illnesses being made by the Epidemiology Section, LID. In addition, 153 cultures were received from other non-study sources -- including 102 from the Clinical Center Laboratory, NIH.

Of the total 499 cultures, 7 showed no growth on subculture, 5 were not streptococci, and 2 are not complete. Of the 485 streptococcal cultures, 65.2% were Group A, 12.6% Group B, 10.3% Group C, 6.8% Group G, 4.1% No Group, 0.6% Group D, and 0.4% Group K. Within 316 Group A strains, non-typable strains made up 30.4% -- followed by Types 4 (13.6%), 42 (12.3%), 28 (11.1%), 12 (9.5%), 25 and 3 (4.4% each), and 19 (2.2%). Miscellaneous types (1,2,5,6,8,18) made up another 6.8% in the aggregate, and strains not typable because of multiple cross-reactions occurred in 4.8%.

Significance to the Program of the Institute:

The data obtained, when correlated with information derived from serology and from viral studies on simultaneously-taken specimens, will allow assessment of the role of streptococci and of various viruses in upper respiratory diseases in civilian populations. Little accurate or culturally-proven information of this sort is available except from military recruit populations. The streptococcal study is but one portion of a multi-pronged investigation into the causes of upper respiratory illnesses occurring both endemically and epidemically, especially in children.

Proposed Course of the Project:

The past year, 1956, was the third year in which extensive bacteriological sampling of upper respiratory illnesses in the Influenza Study population was done. In 1957, this phase of the study is being curtailed, but the data for the 3-year period are currently being analyzed and should be published in due time. In 1957, however, more intensive study of the semi-closed nursery-age population of Junior Village will be done: in addition to illness specimens, all children will be routinely cultured, both bacteriologically and virologically, once weekly. The bacteriology studies, done in this Unit, are being expanded to include the genus Hemophilus, for which purpose an additional bacteriologist GS-7 has been obtained by the Epidemiology Section.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-6ha
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$6,881	$4,640	$11,521	0.4	0.6	1.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. Emily Emmart, NIAMD

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-64
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956.

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Invited paper - "The role of the Public Health Laboratory on the control of streptococcal disease and its sequelae.", presented before Conference of State and Territorial Public Health Laboratory Directors, Atlantic City, N. J., Nov. 11, 1956.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-64-a
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5. Bethesda
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Streptococcal Disease
PROJECT TITLE

7. Dr. Roger M. Cole
PRINCIPAL INVESTIGATOR

8. Edythe J. Rose
OTHER INVESTIGATORS

9. None

10. PROJECT DESCRIPTION

The localization of injected streptococcal hyaluronidase by the fluorescent antibody technique.

Objectives:

To determine the site of deposition of partially purified Group C streptococcal hyaluronidase, after intravenous injection in mice.

Methods Employed:

Described previously in Project Description NMI-69 for December 1954; and in a publication reported in NMI-38, #16, for December 1955 (J. Bact., 70(5):596-607, November 1955).

Major Findings:

Mice, injected intravenously with Group C hyaluronidase prepared as described, were killed within 30 minutes: selected tissues were immediately removed and frozen in isopentane by liquid nitrogen. Tissues were sectioned in the frozen state

Major Findings (continued):

or were paraffin-embedded from the frozen state and then sectioned. The antigen, as localized by its combination with specific fluorescent globulin, has been found in parts of all tissues thus far examined. In kidney, the antigen was thus localized in the nuclei of tubular cells if frozen-sectioned tissue was used, but in the cytoplasm of the same cells if the tissue was first paraffin-embedded. No such differences between frozen and frozen-embedded preparations were noted in other tissues, although there was gradual centrifugal loss of antigen from liver lobules with time after freezing. In liver, the antigen was found in the nuclei of the hepatic cells and in the cytoplasm of Kupffer cells. It was also found in cells of the red, but not white, splenic pulp; in the cytoplasm of syncytial cells of lymphoid tissue; in the cytoplasm of some septal cells of the pulmonary alvioli; in the cytoplasm of ciliated epithelial cells of the bronchi; and in a number of cells of the skin. The major part of this work has been done by Dr. Emily Emmart, NIAMD.

Significance to the Program of the Institute:

Little is known of the localization in the host of streptococcal antigens -- some of which may be important in the delayed and presumably hypersensitive state culminating in rheumatic fever or glomerulonephritis. The present investigation offers a useful experience in a tool for investigation of the present and other antigens in this respect.

Proposed Course of the Project:

It is planned to continue the studies with hyaluronidase, with sacrifice of animals at greater intervals of time after injection. Extension to studies of Group A hyaluronidase, as well as to other Group A antigens, will be possible now that full experience with the technique has been obtained.

Form No. OHP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE--NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-64
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$23,970	$16,164	$40,134	1.3	1.6	2.9

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	

13. BUDGET ACTIVITY:

RESEARCH /X/ ADMINISTRATION ☐

REVIEW & APPROVAL ☐ PROFESSIONAL & TECHNICAL ASSISTANCE ☐

BIOLOGIC STANDARDS ☐

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-64-a
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-65
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of Mycoses and Pathogenic Fungi
PROJECT TITLE

7. Chester W. Emmons, Ph.D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

Some of the studies are similar to those carried on in the Mycology Laboratory at CDC, but these studies were pioneered here. So far as known there is no duplication of the studies in mycology at Rocky Mountain Laboratory.

10. PROJECT DESCRIPTION

A. Epidemiologic studies of mycoses.

Objectives:

To investigate individual cases of mycoses and family epidemics, to determine environmental sources of infection, to apply environmental studies to diagnostic methods, to derive from these studies specific information about the factors determining regional and geographic distribution of the pathogenic fungi and the diseases they cause.

Methods:

Soil samples taken from environments of patients are processed to determine whether fungi are present, the occurrence of the mycoses in animals in the household is looked for, research methods are applied to attempts to remove pathogenic fungi from the immediate environment in which it is found.

Major findings:

We continue to isolate Histoplasma and Cryptococcus from saprophytic sources in the environment of cases studied in collaboration with physicians of the Clinical Center and outside physicians.

Significance:

These environmental studies are important because the systemic mycoses are not contagious and an environmental source of infection is responsible for almost all cases of the deep mycoses. Such studies have led to a much better understanding of the nature and occurrence of the mycoses.

Proposed course:

It is planned to continue these studies indefinitely with special emphasis upon the factors which determine the occurrence of pathogens in soil and organic debris.

B. Experimental chemotherapy of mycoses.

Objectives:

To test the efficacy in experimental mycoses of new drugs and antibiotics as they become available. To evaluate these for eventual clinical use.

Methods:

A standard procedure of producing experimental histoplasmosis, cryptococcosis, coccidioidomycosis and other mycoses has been developed. Inbred white mice are injected intravenously with a carefully measured

inoculating dose and daily treatment is begun on the following day. The procedure gives a regular and reproducable death rate. The day of death is noted, and extension of survival time of treated mice beyond that of a control group of mice is taken as a measure of efficacy. When necessary treated mice are examined by culture to determine whether clearance of organs has occurred.

Major findings:

Many of the drugs tested have been found inactive or toxic. Marked therapeutic effect has been shown for mycostatin and Amphotericin B and effective serum levels for these antibiotics have been determined. Several silver salts were shown to be effective in extending the survival time of mice with experimental cryptococcosis, although mice were not cured with clearing of c.n.s. tissues. Silver was ineffective against histoplasmosis.

Significance:

There is at present no safe and effective treatment against some of the systemic mycoses. Experimental studies of this type must precede clinical trial.

Proposed course:

Experimental chemotherapy will be continued. One new drug shows considerable promise.

C. Effect of Cortisone upon experimental mycoses and subsequent therapy.

Objectives:

To evaluate more systematically than has been done formerly the effect of cortisone upon experimental mycoses and their chemotherapy.

Methods:

Mice exposed to varying dosage schedules of cortisone are given an experimental mycosis according to the standard procedures of this laboratory and then given specific chemotherapy. This project is the responsibility of Drs. Louria and Feder.

Major findings:

Information from this experiment is still fragmentary.

Significance:

It is known that cortisone has a deleterious effect upon the patient and the experimental animal with mycosis. The question of the importance of this effect has repeatedly arison in the Clinical Center.

The information about this effect is scattered and lacks uniformity. The project was undertaken to obtain more uniform and standardized data from a systematic study.

D. Sterilization methods effective against fungi.

Objectives:

To determine whether ethylene oxide is effective in sterilizing hospital equipment contaminated by pathogenic fungi.

Methods:

Filter paper test strips were uniformly contaminated with various pathogenic fungi in both tissue and mycelial phases of growth. A part of the set was given to Mr. Floyd Taylor for test in an ethylene oxide sterilizer. These were subsequently cultured for comparison with test strips kept as controls.

Major findings:

The method is effective. Results have not yet been reported.

Significance:

This method, if effective, will be useful in sterilization of certain hospital equipment.

Proposed course:

The investigation will be discontinued unless the necessity arises for further tests.

E. Studies of unusual fungi appearing in the diagnostic service.

Objectives:

To learn more about unusual fungus pathogens, their geographic distribution, their virulence and pathogenicity.

Methods:

A limited diagnostic service is maintained partly for the benefit of outside physicians who may be induced to refer interesting patients to the Clinical Center, partly because it has for many years supplied the laboratory with interesting and rare pathogens.

Major findings:

As a direct result of this project we have within the past year had an opportunity to study in more or less detail a case of meningitis caused by Sporotrichum, two unique cutaneous and subcutaneous infections caused by Basidiobolus ranarum and Cercospora apii, respectively, (through the collaboration of Dr. Lie and his associates, Djakarta, Indonesia), unusual dermatophytes from Cairo, several interesting instances of environmental exposure to Histoplasma, Aspergillus fumigatus, etc.

Significance:

Inherent in the nature of the mycoses is the occasional occurrence of a mycosis caused by a fungus not usually recognized as pathogenic. The full clinical variation of some of the mycoses is also poorly defined. The diagnostic service provided has been very useful in bringing to the Mycology Laboratory unusual fungi.

Proposed course:

This project will be indefinitely continued.

Form NO. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAIL-65
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$25,452	11,360	36,812	2.0	2.8	4.8

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	3	5	

13. BUDGET ACTIVITY:

RESEARCH [X]
REVIEW AND APPROVAL [ ]
BIOLOGICS STANDARDS [ ]
ADMINISTRATION [ ]
PROFESSIONAL & TECHNICAL ASSISTANCE [ ]

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS NIH, INDICATE SERIAL NO(S):

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-65
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Emmons, C.W. Medical Mycology in the United States. Bull. Internatl. Soc. Human & Animal Mycology. 1:11-13, 1956.

Emmons, C.W. The isolation of Cryptococcus neoformans from pigeon droppings. Therapy of Fungus Diseases. An International Symposium. Ed. T. H. Sternberg and V. D. Newcomer. Little Brown & Co., Boston, 1955.

Emmons, C.W. Medical Mycology. Chap. XI in Gradwohl, Clinical Laboratory Methods and Diagnosis. Ed. 5. Mosby. Vol. II. pp. 1764-1793, 1956.

Emmons, C.W. Review. Guide Pratique de Mycologie Medicale, by Jean Coudert. Mycologia 48:771-772, 1956.

Emmons, C.W. Silver in the treatment of experimental cryptococcosis. Antibiotics and Chemotherapy. 6:598-602, 1956.

Lie-Kian-Joe, Njo-Injo Tjoel Eng, Sratono Kertopati and Chester W. Emmons. A new verrucous mycosis caused by Cercospora apii. In press. Archives of Dermatology.

Emmons, C. W., Lie-Kian-Joe, Njo-Injo Tjoei Eng, A. Pohan, S. Kertopati and A. van der Meulen. Two unusual fungus pathogens of man. In press. Mycologia.

Emmons, C. W. Fungus Infections. In press. Encyclopedia Brittanica.

Emmons, C. W. Epidemiology of the Mycoses. In press. Proc. Sixth Middle East Medical Assembly, Beirut.

Louria, Donald B., Feder, Ned and Emmons, C. W. Amphotericin B in experimental histoplasmosis and cryptococcosis. In press. Proc. Annual Antibiotics Symposium, Washington, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Emmons, C. W. Appointed Consultant to NAMRU-3, Cairo, 1956.

Emmons, C. W. Consultant, National Research Council Committee on Index of Fungus Culture Collections, Quartermasters Depot, Natick, Mass.

Emmons, C. W. Invited paper on Ecology of Histoplasma and other pathogenic fungi, A.P.H.A. meeting, Atlantic City.

Emmons, C. W. Invitation to give a paper before the Isthmian Medical Associa tion, Panama, in February, 1957.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-66
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3.
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Physiological Studies on Pathogenic Fungi
PROJECT TITLE

7. Leo Pine, Ph.D.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION

A. Determination of the Vitamin Requirements of Yeast and Mycelial Phases of Histoplasma capsulatum.

Objective:

To determine the vitamin requirements of the yeast and mycelial phases of Histoplasma capsulatum.

Methods:

Bacteriological and Biochemical Procedures.

Major findings:

No vitamins were found to be required or stimulatory for the mycelial phase at 25 C. At 37 C., the majority of yeast phase strains required thiamine for maximum rate of growth. Biotin was required by one strain and thioctic acid was stimulatory for another. An impaired sulfur metabolism of the yeast at 37 C is suggested.

Significance:

This program is terminated. The results indicate requirements by the yeast phase which have not been recognized and which must be satisfied for satisfactory growth of this pathogen.

B. Conversion of Mycelial Phase of H. capsulatum to Yeast Phase.

Objective:

To determine factors influencing the conversion of mycelial phase of H. capsulatum to the yeast phase.

Major findings:

Conversion of mycelial phase to yeast phase does not merely involve the satisfaction of the growth requirements of the yeast phase and an increased temperature. Results suggest that an initial anaerobic incubation or perhaps binding of an unknown metal by cholation with cysteine may be a primary factor responsible for the initiation of conversion of mycelium to yeast phase.

Significance:

This program is terminated.

C. Carbon Dioxide Fixation in Glucose Fermentation by Actinomyces species L. bifidus.

Objective:

To determine the manner in which carbon dioxide is fixed into the products of glucose fermentation by Actinomyces species and L. bifidus.

Methods:

Bacteriological and biochemical procedures; radioactive tracer techniques.

Major findings:

Although carbon dioxide is fixed into carboxyl groups presumably by the classical Wood-Workman reaction, the above organisms fix carbon dioxide into the methylene carbons of succinic acid. This reaction has not been reported previously.

Significance:

Fixation of carbon dioxide into methylene carbons of succinic acid is an unreported biochemical process and studies are to be done investigating the mechanism by which this process occurs.

D. Physiological and Biochemical Characteristics of Actinomyces species and Lactobacillus bifidus.

Objective:

Comparison of the physiological and biochemical characteristics of Actinomyces species and Lactobacillus bifidus.

Methods:

Bacteriological and biochemical procedures; fermentation analyses.

Major findings:

Although similar qualitatively, the glucose fermentations of Actinomyces species and Lactobacillus bifidus differ quantitatively. Actinomyces ferment glucose forming lactic acid and succinic acids primarily, while Lactobacillus bifidus forms lactic and acetic acids. Formate is also formed by both organisms. Both fix carbon dioxide.

Significance:

The program is terminated. The results further differentiate a saprophytic group of organisms (Lactobacillus bifidus) from potential pathogen (Actinomyces).

Form No. ORP-1
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(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID - 66
SERIAL NUMBER

12. BUDGET DATA

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY '57	$15,650	6,992	22,642	1.0	1.4	2.4

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. Arden Howell, Jr., NIDR

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. _____
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Howell, Arden, Jr, and Pine, L. 1956. Studies on the growth of Actinomyces. 1. Cultivation in a synthetic medium with starch. J. Bacterial., 71, 47-53.

Pine, L. and Howell, A., Jr. 1956. Comparison of Physiological and Biochemical Characteristics of Actinomyces spp. with Lactobacillus bifidus. J. gen. Microbiol., 15, 428-438.

Pine, L. Fixation of Carbondioxide by Actinomyces and Lactobacillus bifidus. 1956. Proc. Soc. Exp. Biol. Med., 93, December.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Senior Research Fellow, Duke University.

Form No. ORP-1 Calenda r Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. MIAID - 67
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
(INSTITUTE OR DIVISION)

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Bacterial & Mycotic Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Investigations on Etiological Agents of Diarrheal Diseases
PROJECT TITLE

7. Dr. A. Elizabeth Verder
PRINCIPAL INVESTIGATOR

8. NONE
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

A. Investigations on Etiological Agents of Diarrheal Diseases.

Objectives:

Investigation of outbreaks of diarrheal diseases from the standpoint of etiology and epidemiology; intensive research is carried out on those outbreaks of unknown etiology or unusual mode of spread.

Methods Employed:

Studies are initiated through the use of conventional techniques for the isolation of potentially pathogenic Enterobacteriaceae and other bacterial pathogens known to cause gastro-enteritis. When rigorous application of these techniques does not reveal the etiology, new methods must be developed and tried. In view of recently reported findings not only potentially pathogenic bacteria but also possible viral and rickettsial agents should be sought.

Appropriate serological methods are employed for the identification and typing of the strains of organisms isolated and for the study of the immunological response of the individuals involved in the outbreak to the agent regarded as the probable etiological agent or as a contributing factor in the illness.

Major Findings:

Unpublished data have been accumulated on outbreaks in which organisms of the Arizona group and the Ballerup-Bethesda group of the Enterobacteriaceae have been isolated from the stools of a sufficiently large number of adults, who were involved to suggest some correlation between their presence and the development of illness. The work with the Arizona group was accomplished during an investigation of an outbreak of food poisoning at Providence Hospital. As far as I know it is still the most clear-cut outbreak in which a strain of the Arizona group appears to be the etiological agent. The most extensive work with the Ballerup-Bethesda group was done in connection with studies on an outbreak of a polio-like illness at Chestnut Lodge, Rockville; our findings suggest some correlation between the presence of strains of this group and the manifestations of the syndrome - the most logical relationship appears to be a common source for these organisms and the etiological agent which was probably a virus although none was isolated with the available techniques. These findings will be reported in conjunction with the viral studies. The exact significance of these organisms as agents isolated from patients manifesting some gastro-intestinal symptoms remains to be established. Spontaneous outbreaks of illness occurring in the study population at Junior Village.

B. L-phase Variants and Pleuropneumonia-like Organisms

Objectives:

To study the characteristics of some L-phase variants and pleuropneumonia-like organisms isolated from man and animals.

Methods:

Bacteriological technics will be used to study the morphology and physiology of the organisms including nutritional and growth requirements, growth in tissue culture, biochemical properties, filterability, immunological reactions and pathogenicity.

Major Findings:

Work was started following the isolation of some cultures from humans. Attempts to study the pathogenicity of those strains for laboratory animals led to the isolation of some animal strains. Studies which have been carried out so far have served primarily to point out the many difficulties with which workers with this group are confronted. Since many laboratory animals harbor morphologically similar strains, satisfactory animals for use in studies on pathogenicity are being sought.

Proposed Course:

The work on sporadic outbreaks is to be terminated as a primary project unless the occurrence of unexpected events or outbreaks prompt reconsideration. Instead a collaborative study will be undertaken with the Sections of Virology and Epidemiology on the role of bacteria as etiological agents.

Significance:

The group of pleuropneumonia-like organisms and the morphologically similar L-phase variants of bacteria have not been studied sufficiently for their importance as infectious agents to be evaluated satisfactorily. Since strains are being isolated in considerable numbers by various workers from both animals and man, their significance as potential pathogens should be appraised.

Proposed Course:

Intensive work on a few strains in proposed so that methods may be obtained for the study of a larger number of strains from various sources.

C. Characterization of Organisms of Genus Pseudomonas

Objectives:

To elucidate the role of organisms of genus Pseudomonas as human pathogens.

Methods:

In vitro studies to detect properties similar to those which characterize known human pathogens of other genera and in vivo work with animals to study their ability to invade tissues and/or their capacity for damaging them.

Major Findings:

Studies on the classification of a large number of strains have given us: 1) biochemical characteristics which permit more accurate separation of the species within the genus as well as identification of an organism as a Pseudomonas and 2) serological types within the large group of Ps. aeruginosa (the most important species involved in production of human illness) so that much more exact identification of strains, isolated from various tissues of an individual and his environment is possible.

Significance:

Ps. aeruginosa is becoming increasingly important as a cause of death. It appears to take the role of an opportunist and invade tissues when the defense mechanisms are altered. We frequently fail to assess the importance of its role as a pathogen especially in infancy and childhood, in debilitated adults, and following shock even in healthy individuals. The organisms are ubiquitous and are found in large numbers in the environment of hospital patients.

Proposed Course:

Studies on classification have been completed and will be made available for use by other laboratories. Additional work will be pursued as far as personnel, space and pressure of work on other projects permits.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-67
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$14,716	$6,447	$21,163	0.8	0.8	1.6

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	1	2	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards & Publications 15. NIAID - 67

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956.

Millican, R. Carl, Rust, John, Worder, Elizabeth and Rosenthal, Sanford.

Experimental Chemotherapy of Pseudomonas Infections. I. Production of Fatal Infections in Cortison-Treated Mice. Antibiotics Annual 1956-1957.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALANDAR YEAR 1956:

NONE

Form No. ORP - 1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 68
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Epidemiology
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Epidemiological Studies of Infectious Diseases
PROJECT TITLE

7. Dr. Joseph A. Bell
PRINCIPAL INVESTIGATOR.

8. Ruth E. Anderson
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION

A. Basic epidemiologic studies of undifferentiated respiratory illnesses generally known as the common cold, catarrhal fever, non-streptococcus sore throat, ARD, and adenoviruses

Objectives:

To discover etiologic agents, methods of identification, modes of spread, and methods for prevention and treatment.

Methods Employed:

Person to person spread viral diseases are of special interest because tissue culture techniques now open the door for intensive

virus study. Acute febrile diseases are being observed in Military Groups (Great Lakes Naval Training Center and Fort Ord, California); in civilian population groups (20,000 persons of Influenza Study, q.v.); in children two months to three years of age (District of Columbia orphanage); in hospital admissions (Clinical Center, D. C. General Hospital, and Children's Hospital); and in such other groups (schools, camps, et al) which come to light through notification by private physicians and health officials. Clinical and epidemiologic data, and many specimens for laboratory study are collected from persons with acute febrile disease and their non-ill associates (controls). These are indexed, coded, and tabulated in a multitude of ways to find groups of cases which epidemiologically appear to be caused by a single infectious agent. Specimens from these cases are then subjected to intensive laboratory study in an effort to find a common agent. When successful, an intensive epidemiologic study is set up to establish etiology and to identify the clinical and epidemiologic characteristics of the disease and to devise methods for control.

Volunteers at the Federal Reformatory in Chillicothe, Ohio, and the State Reformatory at Breathedsville, Maryland, were inoculated with various viruses and other material to better elucidate host parasite relations of human infection, immunity and disease. This limited approach has been particularly profitable in promptly giving answers to important questions which otherwise might take years to obtain.

Major Findings:

The cooperating virus laboratory discovered new viruses of the respiratory tract in 1953 and with the help of the above epidemiological setup identified the group and type characteristics of adenoviruses. In 1954, through the hospital setup, Type 3 adenovirus was found in six cases of acute respiratory disease, some having conjunctivitis. Immediately, an epidemiologic study was arranged which established that a newly recognized disease entity, Pharyngoconjunctival Fever, was caused by Type 3 virus, (Published 3/55 J.A.M.A.) and abundant clinical, pathological and epidemiological information was obtained. This led to studies in volunteers, which developed clinical, epidemiological, and laboratory methods for readily recognizing human susceptibility, immunity, infection and disease. (Published Sept. 1956 Am. J. Pub. Health). An inactivated Type 3 virus vaccine was prepared and shown to protect volunteers against infection and disease. (Published Nov. 1955, J.A.M.A.)

Through studies of cases at the Great Lakes Naval Training Center, adenovirus Types 3, 4, and 7, have been confirmed as a major cause of acute febrile respiratory diseases in military recruits. Thus a polyvalent inactivated adenovirus vaccine Types 3, 4, and 7, has been prepared commercially and given to some 4,000 Naval Recruits who have been studied together with 12,000 placebo inoculated controls to determine effectiveness in preventing the naturally occurring infections and disease in military recruits. A progress report shows that the vaccine gave substantial protection against acute respiratory disease (Type 4). This is very promising

and of happy concern to military medicine. (Published Sept. 1956, J.A.M.A.)

Proposed Course of Project:

The studies of adenovirus vaccine in Navy Recruits being conducted in cooperation with Naval Medical Research Unit No. 4 at the Great Lakes Naval Training Center is finished except for completion of data analysis and further publication of results.

The studies of adenovirus vaccine in Army Recruits being conducted in cooperation with Dr. Edwin H. Lennette and Dr. James O. Culver at Fort Ord, California, are well underway. A study of neutralizing antibody response to varying doses and varying intervals between doses is nearly complete and the trivalent vaccine is being administered to a sample of arriving recruits.

Projects NIAID 69, 70, and 70a relate the status of general population studies.

Further surveys of hospital admissions must await availability of funds, space, personnel, etc.

Further special studies in camps, schools, etc. await opportunities more worthy of study than those now in progress.

Studies in volunteers in prison groups are being continued by Dr. Huebner's unit in cooperation with Dr. Thomas Ward of Lobund Institute, University of Notre Dame, Indiana.

Studies in the District of Columbia Junior Village Nursery are being expanded so that it will be the most intense study of host-parasite-disease-relationship ever conducted. Four Laboratory of Infectious Diseases Units are now cooperating in this study, and others may eventually be involved.

The epidemiologic techniques that have been developed and new laboratory tools available hold promise for further discovery of the cause and clinical epidemiological attributes of other at present undifferentiated respiratory diseases. It is proposed to continue this most promising project for these ends.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-68
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$43,333	$21,186	$64,519	2.7	7.0	9.7

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3	7	10	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. Edwin H. Lennette, Fort Ord, California
Capt. Matthew J. Hantover, MC, USN, Great Lakes, Illinois

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

"Adenoviruses": "Group Name Proposed for New Respiratory-Tract Viruses"

Enders, J.F.; Bell, J.A.; Dingle, J.H.; Francis, Thomas, Jr.; Hilleman, M.R.; Huebner, R.J.; and Payne, A.M.-S.

Science (July 20, 1956) Vol. 124: 119-120

"Efficacy of Trivalent Adenovirus (APC) Vaccine in Naval Recruits-Progress Report"

Bell, J.A.; Hantover, M.J.; Huebner, R.J.; and Moosli, C.G.

J.A.M.A. (Aug. 1[illegible], 1956) Vol. 161: 1521-1525

"Studies of Adenoviruses (APC)* in Volunteers"

Bell, J.A.; Ward, T.G.; Huebner, R.J.; Rowe, W.P.; Suskind, R.G.; and Paffenbarger, R.S., Jr.

Am. J. Pub. Health (Sept. 1956) Vol. 46: 1130-1146

"Continued Observations on Histoplasmin Sensitivity and Calcified Pulmonary Lesions in a Rural Community"

Mattern, C.F.T.; Bell, J.A.; Olson, [illegible]; Emmons, C.[illegible]; and Powell, [illegible]

Public Health Monograph No. 39 PHS Publication No. 465 (Aug. 1956) Issued concurrently with Vol. 71, No. 8 Public Health Reports

17. LIST HONORS AND AWARDS TO PERSONNEL [illegible] TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. ORP - 1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 70
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Epidemiology
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of Viral Agents in Relation to Illness in the General Population
PROJECT TITLE

7. Dr. Alexis Shelokov
PRINCIPAL INVESTIGATOR

8. Dr. Albert M. Lerner, Miss Lotta Li
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

Dr. Habel, NIAID - LID
Dr. Rosen, NIAID - LID

10. PROJECT DESCRIPTION

Objectives:

The objective of this new project, initiated September 28, 1956, is to search for viral agents responsible for, or associated with, febrile illnesses in a large group of families representative of the general population.

Methods Employed:

Approximately 2,500 families in Montgomery County totaling 8,000 individuals are under continuous surveillance by the Epidemiologic Section. A system of case reporting of febrile illnesses previously worked out for epidemiologic studies of the efficacy of

influenza vaccination is utilized to detect the occurrence of such illness in the population group. The field team collects specimens of throat and rectal swabs (other specimens as indicated) on the index case as well as on all the other members of the household available whether well or ill. Follow-up home visits are made as indicated. The swabs are routinely inoculated into tissue culture of monkey kidney, human amnion, and human chorion cells in order to detect viruses possibly possessing unusual cell system predilection. Suckling mice are routinely inoculated with materials from patients suspected of harboring Coxsackie viruses and also from random field specimens which had been found to be negative in tissue culture as an index of the occurrence of viral agents optimally detected in this host. Other diagnostic procedures are performed as indicated, including embryonated egg and adult mouse inoculations, serum neutralization, hemagglutination, and complement fixation reactions. The major study is complemented by short-term investigations of specific outbreaks of infectious diseases and of interesting individual patients with infections possibly due to viral agents. In addition, three specific sub-projects have also been pursued:

1. Viral studies of hospitalized cases of clinically diagnosed primary atypical pneumonia with the staff of the Medical Service, National Naval Medical Center -- Dr. Ralph C. Parker, Jr., Chief, Medical Services, (initiated in September 1956).

2. Further exploration of the usefulness of human placental tissue cells in viral diagnostic work, extending the earlier important findings of the usefulness of human amnion cells to the now successfully employed human chorion cells.

3. Studies of unusual viral agents procured from the Soviet microbiologists. These include strain BBBF said to be the causal agent of "viral chorioencephalitis" and strain F-1, a presumed cause of viral gastroenteritis in the Soviet Republic.

Major Findings:

As the major project was initiated on September 28, 1956, it is too early to discuss findings of possible eventual importance. However, as anticipated, a number of viral agents cytopathogenic for various tissue culture cells and/or for suckling mice have been isolated. Most of these are still under study pending final identification, although a few have been typed as Coxsackie and adenoviruses. It is of interest to note that certain of the agents were detected only by using the amnion or chorion cell cultures. The sub-projects have so far yielded the following results:

1. The feasibility of using chorion as a source of human fibroblasts has been explored and its range of usefulness is being established.

2. A strain designated BBBF which is said to be the cause of

"viral chorioencephalitis" has been found to be closely related, if not indistinguishable, from the virus of lymphocytic choriomeningitis. This is in accord with the Soviet studies. A virus strain F-1 believed by the Soviet workers to be a cause of viral gastroenteritis has been established in suckling mice and is now being classified. Attempts are being made to relate this agent to the occurrence of gastroenteritis.

Significance to Microbiological Research

The project was undertaken in order to explore the possibility of establishing the causal relationship between the occurrence of febrile illnesses and isolation of respiratory and enteric viruses using modern diagnostic procedures now available on a large scale. It is intended to serve as a controlled study of the population group living in their normal environment as differing from a "captive" institutionalized population. In the course of this study viral agents are detected under natural conditions of community living as multiple family units in contact with each other through schools, work, and social intercourse, rather than the close living of a "large family" of inmates in orphanages, prisons, military training camps, etc. Thus, there is an opportunity through using individual and community controls to elucidate not only the possible causal relationship of certain of these viruses in terms of the spectrum of clinical diseases they may produce, but also to study the flow of such viruses through a community in the course of the different seasons of the year, as well as from one year to the next.

This is particularly important today when the virologists have reached the stage of having many viruses "in search of diseases". It has become necessary not only to re-evaluate our concepts of the etiologic role of some of the viruses well known during the past few years, such as certain members of the Coxsackie A and B virus groups, but the entire heterogeneous array of agents of unknown significance, generically designated as orphan or ECHO viruses, demand prompt evaluation.

In the course of these investigations new viruses are likely to be found and new procedures are bound to be established facilitating viral diagnostic work. An example of the latter is to be found in the demonstration of the usefulness of human placental tissues as a source of susceptible tissue culture cells of interesting and possibly extended potentialities.

Proposed Course:

It is expected that this study will continue for a period of at least one year and more likely several years, depending on the eventual opportunity of satisfying the objectives. The major study will be supplemented by concurrent investigations of epidemic outbreaks in the area. Continuous surveillance of school absenteeism in the study areas will be used as an index of prevalent illness in the community.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$29,117	$12,933	$42,050	1.2	2.6	3.8

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	3	5	

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. Ralph C. Parker, Jr., Medical Services, Natl. Naval Med. Center
Dr. Ladd Loomis, NIAMD, NIH

(Use reverse and additional pages, if necessary)

Form No. ORP-1
Calendar Year 1956

Part C: Honors, Awards & Publications

15. NIAID - 70
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. ORP-1

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 70b
SERIAL NUMBER

2. NIAID
INSTITUTE

3. LID
LABORATORY

4. Virus & Rickettsial Diseases
SECTION

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Growth and Metabolism of Rickettsiae
PROJECT TITLE

7. Elizabeth B. Jackson
PRINCIPAL INVESTIGATOR

8. Hope E. Hopps, Joseph X. Danauskas, Joseph E. Smadel
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

Organization of Unit. The Rickettsial Biology and Metabolism Unit of the Virus and Rickettsial Diseases Section, LID-NIAID, was activated late in April 1956. The professional staff of 3 persons (GS-12, GS-9 and GS-6) trained in the techniques of viral and rickettsial research is supported by one glass washer-animal caretaker. The laboratory, occupying 900 square feet on the second floor of Building 3, contains the essential equipment for tissue culture and serologic studies, for work with small animals and embryonated eggs as well as the usual equipment for metabolic studies on microbial agents.

Objectives. The objectives are the elucidation of the nutritional and metabolic requirements for proliferation of the rickettsiae and, conversely, a more extensive study of the mode of action of certain of the inhibitors of rickettsial multiplication. Work of the members of the group before joining the staff of the NIAID provided a model system in tissue culture for the quantitative study of growth of rickettsiae in vitro (Bozeman, Hopps, Danauskas, Jackson and Smadel, J. Immunol. 76:475-488, 1956) and had begun to provide information on both of the listed objectives.

As time permits, other biologic properties of rickettsiae including their toxins will be studied. Furthermore, related problems in the field of viral and rickettsial diseases will be investigated; along these lines may be mentioned the unsuccessful attempts during the summer of 1956 to propagate the agents of Crimean Hemorrhagic fever in monkeys and tissue cultures and the agent of Moldavian sandfly fever in eggs (both strains obtained from Russia).

Methods employed. The ordinary procedures used in a rickettsial research laboratory were employed, i.e., propagation of agents in small animals, embryonated eggs and tissue cultures, microscopic examinations, and simple biochemical and metabolic assays.

Major findings. Earlier observations had indicated that Rickettsia tsutsugamushi increased about threefold each 24 hours when growing in cultures of mouse lymphoblast (MBIII) cells. This slow rate of multiplication has now been encountered when this organism grows in cultures of mouse fibroblast (Earle's L929) cells. Similar findings in two different but related systems strengthen the suggestion that rickettsial multiplication proceeds at a rate infinitely slower than the rates for bacteria and viruses.

The R. tsutsugamushi - L cell system, unencumbered by the complexities of an animal host, has been useful for further studies on the mode of action of chloramphenicol. For the first time infected tissue cultures have been completely freed of rickettsiae by treatment with one of the broad spectrum antibiotics. Chloramphenicol at a concentration of 5 ug/ml in the culture medium promptly suppresses growth of rickettsiae but the microbes remain viable for some time and recover and multiply in the cells unless the antibiotic level is maintained in the culture fluid for several weeks. Cultures treated for 3 weeks are non-infectious for mice and rickettsiae do not reappear when the cultures are maintained for an additional period in normal culture medium without chloramphenicol. All earlier evidence pointed toward the rickettsiostatic effect of broad spectrum antibiotics and "cure" of patients and animals was assumed to depend on immunologic phenomena. Present observations suggest that a rickettsiocidal action of chloramphenicol may be elicited under certain circumstances.

Significance to the program of the Institute. The advent of effective measures for the diagnosis, control and treatment of most of the important rickettsial diseases in man has resulted in a concurrent decline in the number of laboratories throughout the country which are still actively engaged in epidemiological, biochemical and serological studies on this group of organisms; at the NIH this holds true not only for the LID at Bethesda but also for the Rocky Mountain Laboratory at Hamilton. Nevertheless, the rickettsiae remain as a constant threat to the population in terms of epidemic potential, should war or a national disaster occur, and it is important that a small nucleus of workers experienced in rickettsial

diseases be maintained. The unit is capable of transferring its operations from basic research to diagnostic and biological control procedures at short notice; thus, it provides a latent resource to the Institute.

On the positive side, the rickettsiae provide a unique group of microbial agents for investigating problems concerned with intracellular parasitism. Although obligate intracellular parasites, the rickettsiae are large enough to be observed by ordinary microscopy and are capable of certain metabolic activities when placed in selected extracellular environments. It is reasonable that many of the fundamental problems of virus-host cell relationship can be studied most profitably by the use of rickettsia-cell models.

Proposed course of the project: Although the use of purified rickettsial suspensions has added considerably to our knowledge of the enzymatic makeup of the rickettsiae, the infected cell remains the simplest unit for studying these organisms in a dynamic state, i.e., during growth and reproduction. Recent experiments indicate that infected cells can be successfully maintained in Eagle's basal medium and in NCTC-107, both of which are chemically defined. With this system one may now hope to determine the alterations occurring in the host cell as a result of infection and the metabolic factors which are directly essential for growth of the rickettsiae, the latter being accomplished both by the addition of possible required metabolites and by inhibitor analysis techniques.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70b
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$26,075	$11,530	$37,605	2.0	2.0	4.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	2	4	

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID - 70b
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 70 C
SERIAL NUMBER

2. Allergy & Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Virus & Rickettsial Diseases
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Adenovirus Vaccine Studies
PROJECT TITLE

7. Dr. Robert J. Huebner
PRINCIPAL INVESTIGATOR

8. Dr. Wallace P. Rowe, Miss Janet Hartley
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

A. Field trials of polyvalent adenovirus vaccines designed to prevent prevalent respiratory diseases.

Objectives:

To determine the effectiveness of adenovirus vaccines in preventing respiratory illnesses.

Methods Employed:

Polyvalent formalin-inactivated vaccines will be prepared in collaboration with commercial pharmaceutical companies. Vaccines containing types 3, 4, and 7 will be further tested in military recruits, and a vaccine containing types 1, 2, 3, and 5 will be tested in a pediatric population. (See Project No. NIAID - 70 K).

Both the ability to stimulate neutralizing antibody response, and to prevent clinical disease will be studied.

Major Findings:

A monovalent type 3 adenovirus vaccine was earlier shown to be effective in producing antibody responses and preventing experimentally produced illness in volunteers. Commercially prepared vaccines containing types 3, 4, and 7 were tested at the Great Lakes Naval Training Station and were found to be effective in reducing hospital admission rates for respiratory illnesses among vaccinated naval recruits to one-third of the rates in controls.

Significance:

The importance of adenovirus infections in military recruit populations is well established, as well as the frequent occurrence of epidemics of type 3 infection in civilian populations, and suggests that a vaccine containing types 3, 4, and 7 will have application and be used in military recruits and other special groups containing persons of comparable age.

Other adenovirus types occur even more frequently, but chiefly in infancy and early childhood, and are probably responsible for much respiratory disease in this age group. It is possible that the frequency of childhood infections with adenoviruses would justify the use of vaccines in infancy.

Proposed Course of Project:

A polyvalent vaccine containing types 1, 2, 3, and 5 will be tested in infants in conjunction with the respiratory disease studies of the Epidemiological Section of LID, serums will be obtained for study of antibody response, and clinical observation of vaccinated and control groups will be made to evaluate the effect of the vaccine on disease rates. This laboratory will continue to collaborate with groups evaluating trivalent types 3, 4, and 7 vaccines in military recruit populations at the Great Lakes Naval Training Center, (NAMRU #4), and at Fort Ord, California (Virus and Rickettsial Laboratory of the California Department of Public Health).

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70c
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$19,558	$8,671	$28,229	0.4	2.3	2.7

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57		2	2	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Namru #4 - Great Lakes Naval Training Station, Great Lakes, Ill.
Virus & Rickettsial Laboratory, California State Dept. of Health

(Use reverse and additional pages, if necessary)

Both the ability to stimulate neutralizing antibody response, and to prevent clinical disease will be studied.

Major Findings:

A monovalent type 3 adenovirus vaccine was earlier shown to be effective in producing antibody responses and preventing experimentally produced illness in volunteers. Commercially prepared vaccines containing types 3, 4, and 7 were tested at the Great Lakes Naval Training Station and were found to be effective in reducing hospital admission rates for respiratory illnesses among vaccinated naval recruits to one-third of the rates in controls.

Significance:

The importance of adenovirus infections in military recruit populations is well established, as well as the frequent occurrence of epidemics of type 3 infection in civilian populations, and suggests that a vaccine containing types 3, 4, and 7 will have application and be used in military recruits and other special groups containing persons of comparable age.

Other adenovirus types occur even more frequently, but chiefly in infancy and early childhood, and are probably responsible for much respiratory disease in this age group. It is possible that the frequency of childhood infections with adenoviruses would justify the use of vaccines in infancy.

Proposed Course of Project:

A polyvalent vaccine containing types 1, 2, 3, and 5 will be tested in infants in conjunction with the respiratory disease studies of the Epidemiological Section of LID, serums will be obtained for study of antibody response, and clinical observation of vaccinated and control groups will be made to evaluate the effect of the vaccine on disease rates. This laboratory will continue to collaborate with groups evaluating trivalent types 3, 4, and 7 vaccines in military recruit populations at the Great Lakes Naval Training Center, (NAMRU #4), and at Fort Ord, California (Virus and Rickettsial Laboratory of the California Department of Public Health).

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70c
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$19,558	$8,671	$28,229	0.4	2.3	2.7

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57		2	2	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Namru #4 - Great Lakes Naval Training Station, Great Lakes, Ill.
Virus & Rickettsial Laboratory, California State Dept. of Health

(Use reverse and additional pages, if necessary)

Both the ability to stimulate neutralizing antibody response, and to prevent clinical disease will be studied.

Major Findings:

A monovalent type 3 adenovirus vaccine was earlier shown to be effective in producing antibody responses and preventing experimentally produced illness in volunteers. Commercially prepared vaccines containing types 3, 4, and 7 were tested at the Great Lakes Naval Training Station and were found to be effective in reducing hospital admission rates for respiratory illnesses among vaccinated naval recruits to one-third of the rates in controls.

Significance:

The importance of adenovirus infections in military recruit populations is well established, as well as the frequent occurrence of epidemics of type 3 infection in civilian populations, and suggests that a vaccine containing types 3, 4, and 7 will have application and be used in military recruits and other special groups containing persons of comparable age.

Other adenovirus types occur even more frequently, but chiefly in infancy and early childhood, and are probably responsible for much respiratory disease in this age group. It is possible that the frequency of childhood infections with adenoviruses would justify the use of vaccines in infancy.

Proposed Course of Project:

A polyvalent vaccine containing types 1, 2, 3, and 5 will be tested in infants in conjunction with the respiratory disease studies of the Epidemiological Section of LID, serums will be obtained for study of antibody response, and clinical observation of vaccinated and control groups will be made to evaluate the effect of the vaccine on disease rates. This laboratory will continue to collaborate with groups evaluating trivalent types 3, 4, and 7 vaccines in military recruit populations at the Great Lakes Naval Training Center, (NAMRU #4), and at Fort Ord, California (Virus and Rickettsial Laboratory of the California Department of Public Health).

Form No. CRP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NE-II-70c
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$19,558	$8,671	$28,229	0.4	2.3	2.7

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57		2	2	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN THE INDICATE SERIAL NO(S):

Narrow - Great Lakes Naval Training Station, Great Lakes, Ill.
Virus & Rickettsial Laboratory, California State Dept. of Health

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications: 15. NIAID - 70 C
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Bell, J.A., Hantover, M.J., Huebner, R.J., and Loosli, C.G. Efficacy of trivalent adenovirus (APC) vaccine in naval recruits. J.A.M.A., v161, 1521-1525, 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. ORP-1 Calender Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 70 D
SERIAL NUMBER

2. Allergy & Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Virus & Rickettsial Diseases
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of the Human Salivary Gland Virus
PROJECT TITLE

7. Dr. Wallace P. Rowe
PRINCIPAL INVESTIGATOR

8. Miss Janet Hartley, Dr. Robert J. Huebner
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION:

Objectives:

To determine the significance of the human salivary gland (Cytomegalic Inclusion Disease) virus in the production of human disease.

Methods Employed:

Laboratory procedure for isolating and detecting serological responses to the human salivary gland virus have been developed to the point where combined clinical, epidemiological and laboratory studies are now possible. Initially, studies will be done in various pediatric and obstetric groups to determine the frequency of salivary gland virus infection in various types of illness of the

fetus and young infant. Studies will be done to elucidate the natural history of human infection with this agent, including studies of the mechanism of spread of the virus and the frequency of infection in various geographic and economic groups.

Major Findings:

Serologic findings in this laboratory have shown that viruses isolated in three virus laboratories from human salivary glands, tissue of cytomegalic inclusion disease cases, and normal human adenoids, are closely related if not identical agents, and represent the causative agent of human salivary gland inclusions and cytomegalic inclusion disease. In certain general populations, antibodies are very prevalent, indicating that this virus is a widespread parasite of man. Pathologic studies and observations of similar viruses in animals indicate that the virus remains latent in the salivary gland tissue, and that such viruses can become recrudescent, causing serious pathology; the duration of latency has not been determined.

Significance to Microbiological Research:

Study of the salivary gland virus is important for several reasons. Cytomegalic inclusion disease is being recognized with increasing frequency, and represents a severe and often fatal illness of the newborn, as well as an occasional cause of abortion and stillbirth. It supplies an example of latent infection of humans for which there are closely analogous animal infections. The high frequency of infection as demonstrated in serologic and autopsy surveys, the fact that some of these infections are fatal, and the ability of the virus to produce severe pathology in a wide variety of organs, including the brain, liver, pancreas, kidneys, lungs, heart, adrenals, and hematopoietic tissues, make it important to determine the role of non-fatal infections (known to occur, but generally unrecognized during the acute stages) in the genesis of neurological, renal, pulmonary, cardiovascular, and hematologic disease.

Proposed Course of Project:

In collaboration with interested clinical groups, serologic and virus recovery surveys will be employed to study diseases of unknown etiology, and controlled epidemiological studies will be carried to investigate any associations that may be found with various types of unexplained illnesses, particularly the chronic types. Studies will be made of pregnant women to determine the frequency of infection during pregnancy and the frequency with which such infection results in disease of the fetus or newborn.

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(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70d
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$21,510	$9,514	$31,024	0.5	2.5	3.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	3	4	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications: 15. NIAID - 70 D
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Rowe, W.P., Hartley, J.W., Waterman, S., Turner, H.C., and Huebner, R.J. Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. Proc. Soc. Exper. Biol. and Med., 1956, v92, 418-424.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 70 E
SERIAL NUMBER

2. Allergy and Infectious Diseases 3. Laboratory of Infectious Dis.
INSTITUTE OR DIVISION LABORATORY, BRANCH, OR DEPT.

4. Virus and Rickettsial Diseases 5. Location (If other than Bethesda)
SECTION OR SERVICE

6. Cultural, Serologic and physico-chemical properties of the guinea pig salivary gland virus, and comparison of these properties with the human and mouse agents.
PROJECT TITLE

7. Miss Janet W. Hartley
PRINCIPAL INVESTIGATOR

8. Dr. Wallace P. Rowe and Dr. R. J. Huebner
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

Objectives:

1. The use of tissue culture and serological techniques for the isolation, identification, and characterization of the salivary gland virus of guinea pigs. 2. Comparative studies with the species specific viruses producing similar pathology in mice and humans.

Methods Employed:

Isolation and serial propagation of the guinea pig salivary gland virus is carried out in tissue cultures of guinea pig embryo muscle fibroblasts. The virus is obtained from suspensions of submaxillary glands from animals originating in a naturally infected colony. The inclusion body agents obtained are identified by cytologic studies in tissue culture, by histopathologic effects in animals, and complement fixation and neutralization tests with serums from naturally and artificially infected animals. Using tissue culture methods and such standard virological techniques as complement fixation and neutralization tests adapted to surmount the difficulties presented by these three agents, experiments are designed to determine growth characteristics and certain physical properties, and to reveal possible serological relationships between the three species specific viruses.

Major Findings:

Isolation, identification and serial propagation of viruses from guinea pig, as well as from mouse salivary glands, were accomplished, and satisfactory complement fixation and tissue neutralization tests were developed for both the mouse and guinea pig agents, in a manner similar to that used for the human agent. (See Project No. 70-D). Comparison of the three salivary gland disease viruses now available for study revealed no specific serologic relationships. However, remarkable similarities were found in tissue culture growth characteristics and in such properties as ether resistance, pH stability, effects of freezing, and conditions of storage. Furthermore, injection of tissue culture grown mouse and guinea pig agents into the respective species reproduced the long-recognized similarities in pathologic effects. Characteristic generalized cytomegalic inclusion disease was produced in each instance.

Significance to Microbiological Research:

The importance of the human salivary gland virus is discussed in Project No. NIAID - 70 D. Limited understanding of the human salivary gland virus infection necessitates the further accumulation of data concerning this and closely related viruses in other species. Animal transmission experiments with mouse and guinea pig salivary gland agents serve as models useful in clarifying the natural history of this highly interesting group of viruses and promise to enlarge concepts bearing on human infection and disease. The use of tissue culture and serological methods for determining virus infection and development of specific antibody in mice and guinea pigs, provide valuable patterns with application to studies of the human problem.

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(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part II: Budget Data

11. MH-ID-70
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$19,762	$8,791	$28,553	0.5	1.4	1.9

	BUDGETED POSITIONS			PATIENT BED
	PROF	OTHER	TOTAL	
FY'57	1	1	2	

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. OPP-1 Calendar Year 1958

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

PART C: Honors, Awards, & Publications 15. NIAID - 70 E
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1958:

NONE

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1958:

NONE

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 70 F
SERIAL NUMBER

2. Allergy & Infectious Diseases 3. Laboratory of Infectious Diseases
INSTITUTE OR DIVISION LABORATORY, BRANCH, OR DEPARTMENT

4. Virus & Rickettsial Diseases 5. _____
SECTION OR SERVICE LOCATION (IF OTHER THAN BETHESDA)

6. Study of viruses as causes of the common cold and other common respiratory diseases.
PROJECT TITLE

7. Dr. Wallace P. Rowe
PRINCIPAL INVESTIGATOR

8. Dr. Robert J. Huebner, Miss Janet W. Hartley
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

None

10. PROJECT DESCRIPTION:

A. Continuation of studies of the clinical importance of the adenovirus group.

Objectives:

To continue studies of the clinical importance of adenovirus infections. At least ten adenovirus serotypes, most of them characterized in this laboratory, remain to be linked with disease.

Methods:

Laboratory methods for detecting infections with adenoviruses are now well established and will be used for determining the frequency of infection with the different adenovirus serotypes in special study groups, including illness groups. Serological studies

will be conducted to determine the frequency of past infection with the newly recognized types and the age distribution of antibodies to these viruses. Volunteer studies will be carried out to determine the characteristics of experimentally produced illness.

Major Findings:

During the past year, information concerning the clinical diseases caused by adenoviruses continued to increase. The importance of types 3, 4, and 7 in the etiology of acute respiratory diseases in military recruits has been confirmed, and a commercially prepared polyvalent adenovirus vaccine was shown to be effective in reducing hospital admission rates for acute respiratory infections at the Great Lakes Naval Training Center. Volunteer studies with type 7 confirmed its ability to produce disease. Studies of the nursery population at the District of Columbia Junior Village have demonstrated that acute infections with adenovirus types 1, 2, 3, and 5 are endemic and highly prevalent in this population; the type 3 infections were usually manifested by pharyngoconjunctival fever or acute febrile pharyngitis. Infection with types 1, 2, and 5 were often associated with febrile respiratory illness, but further observation and analysis is necessary before conclusions can be drawn as to their etiologic importance in these illnesses. In November, 1955, there was a sharp outbreak of type 2 infection; during this period, there was a significant association between isolation of type 2 and the occurrence of fever. Several other serotypes have been encountered in this population, including types 10 and 13, offering the first opportunity to study their relation to disease.

The typing system for the adenovirus group instituted in this laboratory, has been generally accepted, and for the present, this laboratory serves as the central reference laboratory for the identification and characterization of new types of new strains. Fourteen serotypes from humans have now been established, as well as four serotypes from simian sources. An immunological variant of type 7, called type 7-prime, has been established as a distinct entity; in contrast to type 7, which causes acute respiratory disease in military recruits, type 7-prime appears to be a widely disseminated cause of pharyngoconjunctival fever and conjunctivitis in civilian populations.

Significance to Microbiological Research:

The adenoviruses are widely disseminated, highly prevalent viruses capable of causing a variety of respiratory and ocular illnesses, including acute febrile pharyngitis, tonsillitis, and conjunctivitis. Certain types cause ARD and atypical pneumonia in military recruits, and in the general population other types cause pharyngoconjunctival fever, acute follicular conjunctivitis, and epidemic keratoconjunctivitis. A number of isolations of adenoviruses have been made from infants having fatal infections; their role in producing death must be determined.

While several of the adenovirus types, namely, types 3, 4, 7, and 8, are well established as etiological agents of disease, data to establish other types as pathogenic are still inconclusive. Among these are types 1, 2, and 5, which are widespread in infants and young children, and remain latent in the tonsils and adenoids for prolonged periods after the initial infection. Because of their great prevalence and the feasibility of vaccinating against them, it is important to clarify fully their relation to disease.

Projected Course of Project:

Specimens will be obtained from cases of various types of respiratory and ocular infections, primarily in pediatric populations, and tested for adenoviruses; materials for this study will be obtained from various hospitals in the area as well as from the Junior Village study. This laboratory provides a valuable function as the center for classification of the adenovirus group and as the repository of prototype strains.

B. Studies of respiratory diseases of unknown etiology.

Objectives:

To continue to search for etiologic agents of common respiratory diseases and to evaluate the possible etiologic role of recently discovered agents.

Methods:

Materials from various types of undifferentiated respiratory diseases will be inoculated into tissue cultures of various types and the production of cytopathogenic changes and complement fixing antigens will be looked for.

Major Findings:

In studies of respiratory illness at the D.C. Junior Village, more than 1,000 virus isolations have been obtained, in addition to the several hundred isolations of adenoviruses. Most of these strains appear to fall into the Coxsackie A, or ECHO groups. Analysis of the possible etiologic role in disease of this collection has been hindered by the difficulty in identifying so many isolations. Work is in progress to develop rapid methods for identification of viruses of this type.

An unusual cytopathogenic virus, the Wi-3 strain, originally isolated in this laboratory from a child with an exanthematous disease, has been subsequently encountered in control monkey cell cultures and in a child at Junior Village. A complement fixation test has been developed for testing for antibody in humans.

Agents recovered will be compared with established viruses, and new agents will be characterized. Serologic tests will be made with paired serums of persons with and without respiratory illness, using a number of viruses suspected to be causative agents of respiratory disease, in an effort to determine if a correlation exists between infection with the viruses in question and respiratory illness. Viruses, after careful laboratory study, will also be inoculated into volunteers in an effort to determine their disease producing potentialities.

Significance to Microbiological Research:

The great importance of respiratory infections of unknown etiology in causing human discomfort and economic loss has been sufficiently emphasized that it is not necessary to elaborate here.

Proposed Course of Project:

Clinical materials will be obtained through collaborative studies with the Epidemiological Section of LID and Children's Hospital of Washington, D.C. Volunteer studies will be performed in collaboration with Dr. Thomas G. Ward of the Lobund Institute, and the Patuxent Institution.

A large effort must be expended in developing laboratory procedures and reagents for identifying the many known agents which can be expected to occur in studies of this type. Rapid and simplified methods must also be developed for carrying out large-scale epidemiologic studies of agents of particular interest.

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(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70f
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$23,629	$10,478	$34,107	0.2	2.1	2.3

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57		2	2	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Dr. Thomas Ward, Lobund Institute, Notre Dame University
Children's Hospital Research Foundation, Washington, D. C.
Patuxent Institution, Elkridge, Maryland

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-70 F
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Rowe, W.P., Hartley, J.W., and Huebner, R.J. Additional serotypes of the APC virus group. Proc. Soc. Exper. Biol. and Med. 1956, v91, 260-262.

2. Rowe, W.P., and Huebner, R.J. Present knowledge of the clinical significance of the adenoidal-pharyngeal-conjunctival group of viruses. Am. J. Trop. Med. & Hyg. 1956, v5, 453-460.

3. Cockburn, T.A., Rowe, W.P., and Huebner, R.J. Relationship of the 1951 Greeley, Colorado, outbreak of conjunctivitis and pharyngitis to type 3 APC virus infection. Am. J. Hyg., 1956, v63, 250-253.

4. Enders, J.F., Bell, J.A., Dingle, J.H., Francis, T., Hilleman, M.R., Huebner, R.J., and Payne, A.M.-M. "Adenoviruses": group name proposed for new respiratory-tract viruses. Science, 1956, v124, 119-120.

5. Bell, J.A., Hantover, M.J., Huebner, R.J., and Loosli, C.G. Efficacy of trivalent adenovirus (APC) vaccine in naval recruits. J.A.M.A., 1956, v161, 1521-1525.

6. Bell, J.A., Ward, T.G., Huebner, R.J., Rowe, W.P., Suskind, R.G., and Paffenbarger, R.S. Studies of adenoviruses (APC) in volunteers. Amer. J. Pub. Hlth, 1956, v46, 1130-1146.

7. Rowe, W.P., Seal, J.R., Huebner, R.J., Whiteside, J.E., Woolridge, R.L., and Turner, H.C. A study of the role of adenoviruses in acute respiratory infections in a navy recruit population. Am. J. Hyg., 1956, v64, 211-219.

8. Hartley, J.W., Huebner, R.J., and Rowe, W.P. Serial propagation of adenoviruses (APC) in monkey kidney tissue cultures. Proc. Soc. Exper. Biol. and Med., 1956, v92, 667-669.

9. Huebner, R.J., and Rowe, W.P. Adenoviruses as etiologic agents in conjunctivitis and keratoconjunctivitis. Amer. J. Ophth. (in press.)

10. Rowe, W.P., Huebner, R.J., and Bell, J.A. Definition and outline of contemporary information on the adenovirus (APC) group. Bulletin of the New York Academy of Sciences (Conference on Viruses in Search of Disease). To be published.

11. Huebner, R. J. The virologist's dilemma, Ibid.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID - 70 G
SERIAL NUMBER

2. Allergy & Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Virus & Rickettsial Diseases
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of the viruses as possible etiologic agents in chronic, recurrent, and neoplastic diseases.
PROJECT TITLE

7. Dr. Wallace P. Rowe
PRINCIPAL INVESTIGATOR

8. Dr. Robert J. Huebner, Miss Janet Hartley
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

Objectives:

A. To determine the role of viruses in chronic, degenerative, and recurrent diseases.

Methods Employed:

A variety of viral techniques will be applied to materials from patients with the diseases under study. In addition to conventional virus isolation attempts in laboratory animals and tissue culture, more recently developed techniques such as one developed in this laboratory, namely, cultivation of diseased tissues in vitro to "unmask" latent viruses, will be used. Serologic surveys with known viruses will be carried out to compare the incidence of past infection in various disease groups with that in normal individuals.

Major Findings:

This is a new project and present information is limited to evaluation of the new techniques for demonstrating and "unmasking" viruses.

Significance to Microbiological Research:

The etiology of many chronic, recurrent, and degenerative diseases is obscure, and of the available hypotheses concerning their etiology, hypotheses of virus infection as etiology are worthy of investigation, particularly in such diseases as chronic and recurrent pancreatitis, various forms of cardiovascular-renal disease such as myocarditis and nephritis, and chronic forms of hepatic and neurological diseases. The existence of latent viruses of humans, known to be capable of producing recurrent or chronic forms of illness, such as recurrent herpes, recurrences of varicella in later life as herpes zoster, and chronic forms of infection of tissues with the human salivary gland virus (see Project No. NIAID - 70 D), provides a valuable precedent as well as a guide for searching for these and other viruses in chronic diseases of unknown etiology. While projects investigating viruses as etiologic agents of acute illnesses are available in profusion, and promise to be fruitful, the potential importance of persistent and recrudescent viruses in chronic illness makes it important that the newer techniques available for the study of virus infections should be applied to this area.

Proposed Course of Project:

In collaboration with various local hospitals, specimens, particularly serums and tissues, will be obtained from selected patients. These will be thoroughly tested by the procedures mentioned above. If viruses are recovered, they will be characterized, compared with known agents, and if they appear to be new agents, serological techniques will be developed for the detection of past infection. Serological studies will be designed to determine the relative prevalence of infection in patients with the disease under study, as compared with that in control populations. In addition, similar serological surveys will be conducted with known viruses having tropisms for the tissues involved in the disease in question.

Objectives:

B. To study the role of viruses in the etiology of neoplastic disease.

Methods Employed:

New tissue culture techniques, particularly growth of tissues in vitro in an attempt to demonstrate indigenous viral agents, will be carried out on tumors of humans and laboratory animals. Tissues will also be grown directly in the rat peritoneal cavity by the mod-

ification of the Toolan technique used in this laboratory for study of oncolysis of HeLa cells. Using well studied virus-induced tumors of laboratory animals as models, attempts will be made to employ newer virological techniques, as applied to growth of the tissues in tissue culture and in laboratory animals, in attempts to achieve ready and rapid laboratory identification. Procedures for concentration and purification of these known cancer viruses will also be attempted.

Major Findings:

This is a new project, and preliminary studies emphasize, chiefly, methods for propagating cancer in tissue culture and animals - including irradiated and heterologous hosts.

Significance to Microbiological Research:

The importance of neoplastic diseases and the established viral etiology of a number of animal neoplasms suggest the application of newer research techniques to this problem. The chief hinderance to the study of tumors such as the mouse mammary gland carcinoma is the lack of any ready laboratory method for identification, characterization, and qualitation. Present methods require long-term observation of mice for development of tumors. The inability to demonstrate tumor viruses other than by their tumor-producing effect in the homologous animal, implies that the currently available procedures are of little use in the study of human tumor viruses. However, an in vitro procedure developed for studying animal tumor viruses, could be applied to the human cancer problem, with considerable hope of success. It is significant that recently, two tumor-inducing viruses have been shown to produce recognizable changes in tissue culture, the Rous sarcoma virus producing cytopathogenic effects, and the Shope fibroma virus producing inclusion bodies.

Proposed Course of Project:

Human tumors will be obtained at surgery through the cooperation of various hospitals, tissue cultures will be prepared, and trypsinized suspensions will be inoculated into x-irradiated, Cortisone-treated rats. The cultures will be observed for the appearance of cytopathogenic changes, and fluids will be transferred to other types of tissue culture. The rat-grown tumors will be transferred in rats, and suspensions will be tested in tissue cultures. Viral agents recovered will be characterized and, if possible, identified, and serological surveys will be conducted to detect the frequency of infection in various populations of persons with and without the tumor from which the virus was recovered.

As an example of this type of study, in collaboration with the Laboratory of Biology, National Cancer Institute, the mouse mammary gland carcinoma will be studied as a model of virus-induced tumor; the carcinoma will be grown in tissue culture, cytopathogenic effects or other characteristic cytologic patterns will be looked for, and filtrates will be transferred to tissue cultures of normal mouse tissues. Growth of virus-containing carcinoma cells will be injected into irradiated rats, using the Suskind technique (see Project No. NIAID - 70 H). Tissue culture fluids will be used to immunize animals, and serological tests will be carried out with various tissue culture fluids and tumor suspensions as antigens. Attempts will be made to concentrate the virus in order to demonstrate antigen and to increase the possibility of producing effects in tissue culture or disease in suckling mice. Serological and tissue culture methods, developed in the study of animal cancer viruses, will be applied to human breast cancer and other types of neoplasms.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70g
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$26,132	$11,562	$37,694	0.6	1.5	2.1

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	2	3	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID - 70 G
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 70 H
SERIAL NUMBER

2. Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Infectious Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Virus & Rickettsial Diseases
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Experimental Virotherapy of Cancer
PROJECT TITLE

7. Dr. Robert J. Huebner
PRINCIPAL INVESTIGATOR

8. Dr. Wallace P. Rowe, Dr. R. Gerald Suskind, and Miss Janet Hartley.
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

Objectives:

(1) By means of experimental laboratory studies, to select and, if necessary, to adapt human and animal viruses having as one of their properties, the ability to effectively and rapidly destroy human cancer cells.

(2) To test and evaluate such agents for anti-neoplastic activity on cancer cells grown in tissue culture *in vitro* and as solid tumor masses in experimental animals *in vivo*.

(3) In collaboration with the Surgery Branch and the Cancer Pathology Section of NCI, to attempt evaluation in cancer patients of candidate viral agents selected on the basis of laboratory demonstrated anti-neoplastic activity.

Methods Employed:

a. Laboratory Studies:

Initial efforts are aimed at the development of applicable and practically useful methods for measuring and evaluating anti-neoplastic activity of potential oncolytic viruses. Epidermoid carcinoma of the cervix and such common and numerous human agents as Coxsackie, ECHO, and adenoviruses have been employed in the initial studies. Cancer cells grown on glass offer valuable information concerning cell specificity of viruses, and many agents can readily be screened for cytopathogenic effects in such a system. However, such cells have a highly dependent existence and are easily separated from the glass surface - following which, cell death ensues. Consequently, Toolan's technique for growing human cancer cells in rats has been modified for the purpose of providing rapidly growing healthy solid carcinomas in vivo, suitable for testing and adapting oncolytic viral agents. This latter system provides a milieu for cancer cells and virus, more comparable to the human carcinoma. Viral agents are tested for growth and oncolytic properties in this system and promising agents are passed in series in the in vivo as well as in vitro systems, in attempts to adapt virus for rapid and complete in vivo oncolytic effects.

b. Clinical Studies:

Selected candidate viruses are furnished to the Surgery Branch of NCI for human testing. The viral suspensions are clarified, ether treated, filtered through an 03 Selas, and exhaustively tested for all known human pathogens. Previously untried candidate oncolytic agents are given in small amounts to carefully selected patients who have visible tumors accessible for biopsy and suitable for clinical observation, who are susceptible to the virus, and who volunteer for this type of treatment. Viral activity in the patient, and more specifically in the cancer, is observed through intensive clinical and laboratory surveillance. As in the laboratory studies, initial efforts at virotherapy are confined to epidermoid carcinoma of the cervix. Those agents which propagate readily in the local cervix lesion are studied further, both in the laboratory and clinical study systems. Those producing definite oncolytic effects are, of course, studied most intensively.

Major Findings:

a. Laboratory Studies:

(1) A technique was developed for producing solid tumors with tissue culture grown human epidermoid carcinoma cells (HeLa and KB cells) in the rat peritoneal cavity. Rapidly growing tumors (reaching 20 to 30 grams in weight) are produced in sufficient numbers to permit a) screening surveys for potential oncolytic agents, b) adaptation efforts on many human and animal viruses, and c) controlled experiments quantitatively evaluating oncolytic viral activity.

(2) At least five viruses (Coxsackie B and ECHO viruses) have been adapted by serial passage to produce rapid and, in many instances, complete destruction of the tumor (as evaluated grossly and histologically). This acquired property of the viruses was shown to be dependent on in vivo selection of viral progeny, and was shown not to be acquired by in vitro (tissue culture) passage in the same cells. Indeed, the in vivo oncolytic property of present adapted viruses was shown to be lost on subsequent in vitro tissue culture passage.

(3) Many hundreds of solid HeLa and KB rat tumors have been partially or completely destroyed by 3 separate Coxsackie B viruses in less than 6 days, during periods of rapid, vigorous growth of tumor tissue. This oncolytic property increased in total effectiveness and became more rapid in advanced passages. The sixteenth in vivo rat passage destroyed large healthy tumors in 3 days.

(4) Both the HeLa-rat tumor technique and the evaluation of tumor destruction by virus have been reduced to comparatively simple laboratory routines that can be carried out by capable high-level technicians. Evaluation of oncolytic viral activity is based on visual scoring of gross appearance and on weights of treated tumors as compared to controls. The gross evaluations of virus effects have been further evaluated in terms of extensive histologic studies of oncolytic effects by Dr. Robert Love, NCI, and a close correlation found.

(5) Histologic and cytologic findings reveal that the "adapted viruses" produce characteristic reproducible "viral" effects and death of cancer cells, occurring initially in the well-nourished peripheral tumor areas; effects often demonstrable within 48 hours after injection. These "viral" effects are easily distinguishable from non-specific central necrosis. Unadapted virus of the same strains (same stem passage) grows to high titers in the tumors (10^{-7} to 10^{-9}) but does not produce gross oncolytic effects, and does not interfere with tumor growth, the latter revealing little or no histologic evidence of tumor cell destruction.

b. Clinical Studies:

(1) Tests of Coxsackie Viruses: Although plans and protocols for testing tumor adapted viruses are available, work in this area is only beginning. At present, only one patient has been injected with Coxsackie B3 virus. Virus (2 ml., TCD 10^{-5}) was injected directly into the epidermoid carcinoma presenting in the vagina. Results are incomplete, but luxuriant growth of virus in the local tumor is known to have occurred.

(2) Tests of Adenoviruses: The data previously acquired on studies of 34 carcinoma cases was analyzed and reported. Although definite oncolysis, attributable to virus activity, occurred, tumor destruction in all cases was incomplete. Histologic studies of these cases, completed by the Cancer Pathology Section of NCI, did not reveal a specific pattern of destruction distinguishable from non-specific central necrosis. The precise site of viral activity, therefore, was not determined.

Significance to Microbiological and Cancer Research:

The possibility of inducing extensive destruction of human tumor cells growing in vivo by prevalent, non-fatal, human viral agents, has been demonstrated both in the laboratory animal and in man. The large-scale availability in the laboratory of a reproducible human cell type carcinoma grown in rats, allows for more effective and extensive screening of anti-neoplastic agents, both chemotherapeutic and viral. The fact that common viral agents having cytopathogenic effects in tissue culture, do not generally destroy the same cells growing in a solid milieu, is quite significant. More significant is the fact that the application of the commonly used virologic procedure of "adaptation" by serial passages resulted in the selection of virus progeny capable of rapid, frequently total, destruction of a solid tumor in vivo. Candidate agents having this property theoretically offer considerably more promise in virotherapy of the human tumor than agents lacking this property (such as the adenoviruses and unadapted Coxsackie viruses).

Future Course of Project:

(1) Adaptation of additional viruses having oncolytic activity in vivo.

(2) Using "plaque" and limiting dilution technique, adapted viruses having oncolytic activity in vivo will be segregated into clones having a more homogeneous population of oncolytic particles--being "fixed" for this property. Further selection by additional passage in tumored rats and tissue culture will be aimed at obtaining highly oncolytic agents with lessened pathogenic capacity.

(3) Adequate tests for candidate oncolytic viruses in human cases of carcinoma are planned. Viruses recovered from tumor tissue as well as from other sites in the body will be studied and compared for oncolytic and pathogenic activity. Accentuation of desirable traits will be attempted if necessary, by selective serial passage of the virus progeny in human cases, following the pattern already set for laboratory studies. However, it is important to point out that the selection of patients, clinical care, and clinical evaluation of the human studies is the responsibility of the clinical laboratory of the National Cancer Institute.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70h
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$71,035	$31,434	$102,469	1.7	4.3	6.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	4	6	

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Surgery Branch and Chemical Laboratory, NCI
Cancer Pathology Section, NCI

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID - 70 H
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Smith, R.R., Huebner, R.J., Rowe, W.P., Schatten, W.E., and Thomas, L.R. Studies on the use of viruses in the treatment of carcinoma of the cervix. Cancer. To be published.

Suskind, R.G., Huebner, R.J., Rowe, W.P., and Love, R. Studies of viral agents oncolytic for human tumors in a heterologous host. The oncolytic effect of Coxsackie B viruses. Proc. Soc. Exper. Biol. and Med. (in press)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 70 I
SERIAL NUMBER

2. Allergy & Infectious Diseases 3. Laboratory of Infectious Diseases
INSTITUTE OR DIVISION LABORATORY, BRANCH, OR DEPARTMENT

4. Virus & Rickettsial Diseases 5.
SECTION OR SERVICE LOCATION (IF OTHER THAN BETHESDA

6. Development and Application of Techniques
PROJECT TITLE

7. Dr. Leon Rosen
PRINCIPAL INVESTIGATOR

8. Dr. Robert J. Huebner, Dr. Wallace P. Rowe, Miss Janet W. Hartley, & Richard Lynt
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

Development and application of techniques for rapid identification and characterization of numerous, newly recognized viruses.

Objectives:

To devise simplified, rapid, and generally applicable techniques for identifying newly isolated virus agents and classifying them within proper generic categories.

Methods Employed:

Tissue Culture - Attempts to characterize and immunologically identify viral agents will be made chiefly in tissue culture. Tissue culture neutralization tests are presently used for identification of some 40 or more separate viruses falling within polio, APC, ECHO, Coxsackie and exanthemata viral families. Since the Coxsackie A viruses, however, cannot be propagated in tissue culture, methods for working in suckling mice will also be necessary. Similarly, certain other viruses that grow best in chick embryos will be dealt with in this medium. Prototype antisera are generally prepared in rabbits, guinea pigs and mice and research efforts will be directed towards simplifying the long-term procedures now necessarily used for identification purposes. Thus, multiple sera prepared from agents falling in the same family groups, are pooled and their efficiency in detecting representatives of that family determined. Other possible differentiating characteristics such as complement fixing antigens, hemagglutinins, growth characteristics, and tropisms for certain tissues are examined with the purpose of devising short cuts to a most necessary objective, namely, quick identification of viruses.

This unit also collaborates with other organizations such as CDC, NFIP, and WHO in their efforts to solve problems of rapid and practicable virus identification.

Major Findings:

During the year, several leads have been developed which promise to be useful for the rapid identification of viruses.

It has been found that antisera prepared in mice apparently react in a type-specific manner in the complement fixation test in the case of the Group B Coxsackie viruses and in the case of at least some of the ECHO viruses. This has been previously found to be true by this laboratory with the Group A Coxsackie viruses.

In regard to the neutralization tests, a schedule for immunizing rabbits has been found which produces antisera of very high titer against the Group B Coxsackie and ECHO viruses. Methods for quick identification of such common viruses as herpesvirus and vaccinia have been developed in tissue culture. A method has also been developed by which vaccinia virus can be rapidly identified by a hemagglutination-inhibition technique.

Significance to Microbiological Research

Those viruses which produce a suitable complement fixation

antigen can be typed by a simple complement fixation procedure instead of a more laborious neutralization test. By using high-titered serum for identification procedures, many time-consuming titrations of viruses and much expense can be eliminated. Also, pooled sera representing groups of viruses can be more readily prepared.

Proposed Course of Project:

It is proposed to continue the investigation of the possibility of using mouse and guinea pig sera for the complement fixation typing of viruses. Other virus groups (such as the adenoviruses) will also be studied to determine if they produce type-specific antisera in mice.

Additional experimental work will also be undertaken in an attempt to find further means of shortening the identification procedure for the prevalent viruses.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIALL-7041
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$26,962	$11,922	$38,884	0.5	1.9	2.4

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57		2	2	

13. BUDGET ACTIVITY:

RESEARCH	X	ADMINISTRATION	
REVIEW & APPROVAL		PROFESSIONAL & TECHNICAL ASSIST-ANCE	
BIOLOGICS STANDARDS			

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN PHS INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. CRP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

PART C: Honors, Awards & Publications 15. NIAID - 70 I
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956

Rowe, Wallace P., Janet W. Hartley, and Robert J. Huebner: Additional Serotypes of the APC Virus Group. Proc. Soc. Exp. Biol. and Med., 1956, 91, 260-262.

Hartley, Janet W., Robert J. Huebner and Wallace P. Rowe: Serial Propagation of Adenoviruses (APC) in Monkey Kidney Tissue Cultures. Proc. Soc. Exp. Biol. and Med., 1956, 92: 667-669.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Form No. CRP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet 1. NIAID - 70 j
SERIAL NUMBER

2. Allergy & Infectious Diseases 3. Laboratory of Infectious Diseases
INSTITUTE OR DIVISION LABORATORY, BRANCH, OR DEPARTMENT

4. Virus & Rickettsial Diseases 5.
SECTION OR SERVICE LOCATION (IF OTHER THAN BETHESDA)

6. Laboratory and Epidemiologic Studies of Viral Diseases in Infants and Early Childhood.
PROJECT TITLE

7. Dr. Leon Rosen
PRINCIPAL INVESTIGATOR

8. Dr. Robert J. Huebner, Dr. Wallace P. Rowe, Miss Christine Cumming, & Richard Lynt
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

NONE

10. PROJECT DESCRIPTION:

In collaboration with the D.C. Welfare Department, Junior Village, the Epidemiology, Bacterial and Mycology Sections of LID: this unit is engaged in an intensive study of the occurrence of prevalent viruses in an orphanage study population.

Objectives:

To determine the etiology, sources of infection and other characteristics of undifferentiated, poorly understood diseases of presumed viral etiology, and to determine the roles of certain newly discovered groups of viruses as agents of disease.

Methods Employed:

The principal population group under study is an orphanage nursery in the District of Columbia, whose average population is approximately 60 children between the ages of 6 months and 3 years. The turnover of this population is relatively rapid, and as a result, there is a great deal of illness throughout the year.

Rectal temperatures of each child are obtained twice a day, and the entire group is observed and provided medical care by a full-time pediatrician.

Throat and anal swabs are obtained routinely once a week from all the children, and additional specimens are obtained when indicated. Serum specimens are obtained on admission and on discharge and at various intervals in between.

The throat and anal specimens are tested in various tissue culture systems, and where indicated, in animals. The sera are used for antibody studies.

Finally laboratory data are correlated with clinical and epidemiologic information in an effort to determine virus-disease relationships.

Major Findings:

Viruses of several different groups were found to be extremely prevalent in this population. Enteric viruses of the poliomyelitis-Coxsackie-ECHO groups are prevalent at all times of the year. Many isolates of these groups have not as yet been classified because of the technical problems involved (over 40 distinct viruses in these groups have already been described). However, at least some of these viruses appear to be responsible for disease in this population.

Adenoviruses, types 1, 2, 3, and 5 are also common in this population and type 10, and at least one heretofore unknown type, have also been found. Adenovirus type 3 appears to be associated with a relatively severe illness in this population. Disease relationships appear to exist with the other adenovirus types but these data have not, as yet, been fully evaluated.

In addition to the two large groups of viruses mentioned above, other viruses have also been encountered, and are under study. Within the last year a total of more than 300 adenovirus and 1000 enteric virus isolates have been obtained from this population, however, many of the isolates represent reisolations of the same agent persisting for several weeks or more in throat or anal secretions.

Significance to Microbiological Research:

This project represents a somewhat unique and very intensive effort to acquire a more complete understanding of the virologic experiences and the disease consequences of such experiences in infants and young children having high rates of exposure to many different viruses. The virus experience of infants observed over long periods added to the information also being collected concerning bacterial infections should clarify considerably the practical future steps necessary for prevention of the common undifferentiated illnesses of childhood.

Proposed Course of Project:

Experimental vaccines prepared against adenoviruses, Coxsackie and ECHO viruses which commonly and predictably occur in the study population will be evaluated in accordance with agreements with the responsible medical authorities of the Welfare Department. Similarly the effectiveness of antibiotics both as therapy and as prophylactic measures will be evaluated in terms of possible modification of both bacterial and viral illness.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-70j
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$63,418	$28,062	$91,480	0.3	5.0	5.8

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1	5	6	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

PART C: Honors, Awards & Publications 15. NIAID - 70 j
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956.

In Press

Rowe, Wallace P., Robert J. Huebner and Joseph A. Bell: Definition and Outline of Contemporary Information on the Adenovirus (APC) Group. Monograph of the New York Academy of Sciences Conference on Viruses in Search of Disease.

Huebner, Robert J.: The Virologist's Dilemma, Ibid.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-71
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Administration and Research Planning and Coordination
PROJECT TITLE

7. Dr. Willard H. Wright
PRINCIPAL INVESTIGATOR

8. Dr. G. Robert Coatney
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

This project furnishes certain technical, supervisory, and administrative services to all research projects in the Laboratory, as follows:

(a) Over-all planning of research, the coordination of research activities in the various Sections of the Laboratory, and the direction of Foreign Field Stations.

(b) Integration of Laboratory research activities with clinical studies of the Clinical Center.

(c) Supervision over personnel, maintenance, travel, and correspondence.

(d) Requisitioning and supervision of equipment and supplies.

(e) Preparation of reports, budget estimates, and exhibits and other public relations materials.

(f) Maintenance of reference library, reprint files, card catalogues, and specimen collections.

(g) Consultatory services to individuals, commercial organizations, nongovernmental organizations, and Government agencies including liaison activities with other branches of the Service.

(h) Reviewing, editing, and revising scientific and technical reports and manuscripts.

Research projects are reviewed quarterly. If necessary, they are discontinued or revised in accordance with current developments and needs. Some changes have been made in the research program of the Laboratory and some activities directed into new channels. Continued emphasis is being placed on the more fundamental aspects of problems in tropical medicine, such as the metabolic requirements of parasites and the chemistry of their metabolism, the physiology of parasites in relation to their disease-producing proclivities, the mechanism of drug action on parasites and the inhibiting effect of antimetabolites, the employment of the newer techniques of fluorescent and radioactive isotope tagging in basic studies in immunology, virus-vector-tissue relationships, and the carriage of viruses by parasites.

Among research projects initiated during the year were studies on vectors of tropical arthropod-borne viral diseases and the pathogenesis of schistosomiasis with particular reference to hepatic lesions. An expansion of the use of germ-free animals in research on broad and specific problems in virulence, immunity, and pathogenesis was initiated in the Laboratory at Bethesda.

Form No. ORP-1
October 1956
(Attachment 1)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-71
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$42,332	$11,750	$54,082	3.0	4.0	7.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	4	4	8	

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NI-ID-71
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956

Newton, W. L. and Wright, W. H.: The occurrence of a dog filariid other than Dirofilaria immitis in the United States. J. Parasitol., 42, 246-258, June 1956.

In Press

Coatney, G. R.: Introduction of Leon Herbert Schmidt as the Charles Franklin Craig Lecturer. Am. J. Trop. Med. & Hyg.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Dr. W. H. Wright

Elected a member of the Executive Committee of the Tropical Medicine Association of Washington at its Organizational Meeting, January 20, 1956.

Elected to serve on the nominating committee of the Gorgas Memorial Institute for its annual meeting.

Reappointed Professorial Lecturer in Medical Zoology, School of Medicine, The George Washington University.

Dr. G. Robert Coatney

Awarded Alumni Merit Award by Iowa State College.

Elected Vice President of the American Society of Tropical Medicine and Hygiene.

Reappointed to the Expert Panel on Malaria of the World Health Organization.

Reappointed Visiting Lecturer in Tropical Public Health, Harvard School of Public Health.

Reappointed Visiting Professor in the Department of Preventive Medicine and Public Health, Howard University, School of Medicine.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-72
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Foreign Field Stations
PROJECT TITLE

7. Dr. Willard H. Wright
PRINCIPAL INVESTIGATOR

8. Dr. G. R. Coatney, Dr. E.G. Berry, Dr. C.G. Dobrovolny
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

At the request of U.S. Government agencies or other agencies, the Laboratory of Tropical Diseases is engaged in cooperative research projects in various foreign areas as indicated below. On some of these projects, the Laboratory furnishes the personnel while salaries and other costs are assumed by the cooperating agency or agencies. In other cases, the Laboratory maintains the salary of the individual and the other agency or agencies assume all other costs, including travel. These foreign research projects are of advantage in a number of ways. They furnish opportunities for contacts with foreign investigators; they provide facilities for Laboratory personnel to gain valuable experience with tropical diseases in an endemic area; and more importantly they offer the means for the development and exploitation in the field of the results of basic laboratory investigations.

Dr. Elmer Berry returned to Cairo, Egypt in May for two months, to inspect the snail control pilot project of which he had been Director, and which was being carried out jointly by the International Cooperation Administration, the Egyptian Ministry of Health, and this Laboratory. Dr. Charles Dobrovolny continued his assignment to the Rio de Janeiro Office of the

Pan American Sanitary Bureau as expert consultant on the Schistosomiasis Control Program of the Brazilian Government.

Project Subtitle:

Control of Snail Intermediate Hosts of the Human Schistosomes in Egypt

Objectives:

To carry out pilot studies on chemical control of snail intermediate hosts to reduce schistosome infection in man.

Methods Employed:

This project was instituted in July, 1954 for the purpose of evaluating the efficacy of sodium pentachlorophenate, a new molluscacide, for the control of the molluscan intermediate hosts of the human schistosomes in Egypt. The area selected was that of Waraq el-Arab, some 10 kilometers northwest of Cairo, and comprising approximately 40 square miles. Irrigation water is supplied from the Nile through 184 kilometers of canals and drains. After initial snail surveys and examinations to determine the incidence of schistosome infection of school children in the area, the first application of sodium pentachlorophenate was made on October 20, 1954. Two and one-half tons of the chemical were required to provide a residual of 10 ppm in the main intake, the Sawahil Canal, which is 24 kilometers in length. An additional 3 1/2 tons were required to treat the remaining 160 kilometers of secondary and tertiary canals and drains. Some of the heavy vegetation was removed from drains by means of a steel basket operated from a tractor. Certain areas in which it was not feasible to remove vegetation were sprayed with a solution of the chemical by means of a fire hose. Following the initial application, all snails were destroyed within the area. The area was retreated on March 17 and 18, and October 6, 1955, because of snails and snail eggs washed in from the Nile.

In 1956, a few specimens of the vector of Schistosoma haematobium were first encountered following the reopening of the canals, and an application of sodium pentachlorophenate was made. A second treatment, utilizing 5,000 pounds of chemical, was necessitated by the fact that course alterations and the cleaning of other canals brought infested debris into the test area.

Major Findings

Sodium pentachlorophenate has continued to prove to be an extremely useful chemical for snail destruction under conditions in Egypt. The stability of the chemical in the highly turbid Nile water far exceeded expectations, since it maintained its original residual of 10 ppm. after being carried up to 30 kilometers and was still present in certain drains in this concentration six weeks after application. A thorough search for living specimens of the snail host of Schistosoma mansoni failed to reveal any in the test area. Results of the annual survey of schistosomiasis in school children in the Waraq el-Arab area are not yet reported, but it is expected that the past experience of reduced incidence and lack of transmission within the area will continue.

Significance to the Program of the Institute:

Schistosomiasis constitutes the major public health problem in Egypt. Egyptian authorities estimate that it is responsible for a loss of 33 per cent in the productivity of the country. Prior to the demonstration discussed above, efforts to control the disease by various means over a period of years had not resulted in any great progress. More efficient methods are needed for treatment of infected individuals and for the destruction of the snail intermediate hosts. It is believed that the present pilot experiment has demonstrated the value of this new molluscacide in control of the disease.

Proposed Course of Project:

Effective July 1, 1956, the Waraq el-Arab snail control project was transferred to the Ministry of Public Health of Egypt under whose full control it will continue to be evaluated. LTD personnel will provide occasional inspection and consultative services, with the International Cooperation Administration supplying the necessary funds.

Project Subtitle:

Studies on the Chemical Control of Molluscan Intermediate Hosts of Schistosomiasis in Brazil

Objectives:

To conduct field trials with newer molluscacides for the elimination of molluscan intermediate hosts of human schistosomiasis and to act as Special Consultant for the over-all anti-schistosomiasis program.

Methods Employed:

Sodium pentachlorophenate and other molluscacides are assessed as to effectiveness in controlling schistosomiasis in various areas in Brazil. Efficacy in eradicating or reducing snail populations, duration of effect, changes in incidence of schistosomiasis, and cost and method of application are used as criteria. Eradication of the disease is attempted in small, isolated foci.

Major Findings:

Sodium pentachlorophenate is an extremely useful compound for killing vectors of schistosomiasis in Brazil. Experiments indicate that this chemical gives best results, in general, by the insertion of briquettes or the use of the drip method directly into the stream, and can be applied at a cheaper rate than copper sulphate, under Brazilian conditions.

As a result of the aid and information furnished by the project, Brazil has now undertaken a large-scale control program using NaPCP supplemented by treatment of infected people, education in principles of sanitation, and by providing additional sanitary facilities.

Significance to the Program of the Institute:

Schistosomiasis has been uncontrolled in Brazil prior to this project because no effective control methods were known. As of now, however, effective snail control is possible and at a reasonable cost.

Proposed Course of Project:

This project is to be discontinued as of the end of Calendar Year 1956.

Form No. ORP-1
October 1956
(Attachment 1)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data:

11. NIAID-72
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,435	$4,000	$19,435	1.0		1.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1		1	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

For foreign Field Stations studies, cooperation in the form of funds for travel was received from Pan American Sanitary Bureau and in the form of facilities and technical assistance from the Ministry of Education and Health of Brazil.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-72
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Olivier, L.: Observations on vectors of schistosomiasis mansoni kept out of water in the laboratory. I. J. Parasitol., 42, 137-146, April 1956.

Olivier, L. and Barbosa, F.S.: Observations on vectors of schistosomiasis mansoni kept out of water in the laboratory. II. J. Parasitol., 42, 277-286, June 1956.

Olivier, L.; Barbosa, F.S.; and Coelho, M.V.: La influencia de la infeccion con Schistosoma mansoni en la supervivencia del Australorbis glabratus. Bol. Ofic. San. Panamer., 40, 416-421, May 1956.

Olivier, L. and Schneiderman, M.: A method for estimating the density of aquatic snail populations. Exper. Parasitol., 5, 109-117, March 1956.

Dalmat, H.T.: Proposed determination under the plenary powers, of the authorship of, and of the original reference for, the name "Filaria volvulus" (class Nematoda) and proposed validation under the same powers of the emendation from "volvulas" to "vulvulus" of the specific name of this species. Bull. Zool. Nomencl., 12, 86-89, June 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-72
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Olivier, L.: Observations on vectors of schistosomiasis mansoni kept out of water in the laboratory. I. J. Parasitol., 42, 137-146, April 1956.

Olivier, L. and Barbosa, F.S.: Observations on vectors of schistosomiasis mansoni kept out of water in the laboratory. II. J. Parasitol., 42, 277-286, June 1956.

Olivier, L.; Barbosa, F.S.; and Coelho, M.V.: La influencia de la infeccion con Schistosoma mansoni en la supervivencia del Australorbis glabratus. Bol. Ofic. San. Panamer., 40, 416-421, May 1956.

Olivier, L. and Schneiderman, M.: A method for estimating the density of aquatic snail populations. Exper. Parasitol., 5, 109-117, March 1956.

Dalmat, H.T.: Proposed determination under the plenary powers, of the authorship of, and of the original reference for, the name "Filaria volvulus" (class Nematoda) and proposed validation under the same powers of the emendation from "volvulas" to "vulvulus" of the specific name of this species. Bull. Zool. Nomencl., 12, 86-89, June 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

NONE

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-73
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Chief
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Germ-free Animal Studies
PROJECT TITLE

7. Dr. Willard H. Wright
PRINCIPAL INVESTIGATOR

8. Dr. M. L. Newton, Mr. B. F. Phillips, Miss A. M. DeLeva
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

Objectives:

To investigate specific aspects of parasitic and infectious diseases in the germ-free animal; for example, to evaluate the pathogenicity and invasiveness of Entamoeba histolytica in animals without concomitant bacteria. To conduct basic studies in immunology, chemotherapy, and other biological phenomena for which the germ-free animal might prove an invaluable tool.

Methods Employed:

Germ-free young are delivered from conventional animals by Caesarian operation into sterile rearing units; fertile fowl eggs are brought into such units through germicidal traps. Food and equipment are brought in following sterilization by steam under pressure in attached claves; inocula, perishable chemicals, etc. are brought in via

the germicidal traps in sealed ampoules. Experimental procedures and techniques depend upon the particular study involved and generally follow those used with conventional animals except as limited by germ-free maintenance and sterilization requirements. For example, germ-free guinea pigs are inoculated intracecally inside the unit with specially-prepared amoebic material which is brought in via the germicidal trap.

Major Findings:

In the experimental work on pathogenesis of E. histolytica, conducted in germ-free guinea pigs by LTD personnel at LOBUND, it has been found that under a number of circumstances the amoeba may establish itself in the intestinal tissues in the absence of concomitant bacteria. These factors were first found in autoclaved preparations of conventional guinea pig feces. It was found that once amoebic infection was established in the tissues, the supplementation of the diet with the fecal preparation could be discontinued; amoebae were found in lesions up to 60 days after discontinuance. Mechanical injury to the gut wall, done by puncture wounds with a needle, resulted in 1 infection among 9 germ-free animals thus tested. In other experiments, in which sodium thioglycollate or cysteine were administered with the amoebic inoculum, 1 of 5 animals given the former and 3 of 6 given the latter showed tissue invasion by the amoebae. These findings suggest that the greater ability of amoebae to produce lesions when they are associated with bacteria may be due to better survival of the protozoa in the lumen. When added substances produce conditions in the cecal contents which provide some of the factors supplied by the bacteria, amoebae may survive and invade the tissues without concomitant bacteria.

A germ-free program was initiated at NIH late in the summer. Since that time, a limited staff has been trained, some equipment has been assembled, and the first germ-free guinea pigs on the reservation have been produced. At the present time, some of these animals are almost a month old. An experiment to determine whether Trichomonas vaginalis can survive in the germ-free guinea pig (T. hominis has been shown to do so), as a basis for studies on pathogenicity variation in strains without concomitant bacteria, has been initiated.

Significance to the Program of the Institute:

Germ-free animals would seem to provide a vast potential for expanding knowledge in such areas as natural and acquired immunity, allergy, and pathogenesis and course of infection in diseases produced by individual and combinations of organisms. The studies on amoebiasis in germ-free animals, besides providing information on the invasiveness of the amoeba and the pathogenesis of the disease, can furnish insight into related problems of infections produced by other organisms. If T. vaginalis will grow in the germ-free animal a means may be at hand for answering such questions as whether the variation in severity observed in cases of vaginitis is associated with differences in virulence of the trichomonad or is due primarily to the accompanying bacterial flora.

Also, the techniques and skills developed in these various studies provide the basis for broadening the application of the germ-free animal to other fields of study.

Proposed Course of Project:

The studies on amoebiasis in germ-free guinea pigs will be continued and broadened to include mechanisms of pathogenesis and variation in virulence. Attempts to study T. vaginalis in the germ-free animal will be pursued. If facilities are increased, effort will be made to rear other species of animals germ-free. It is expected that the various germ-free activities of LTD will be grouped together under a single Section on Germ-free Animal Studies.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID-73
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

In Press

Phillips, B. P.: Parasitological survey of LOBUND germ-free animals. In LOBUND Report No. 3.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1
October 1956
(Attachment 1)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-73
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$73,861	$25,950	$99,811	3.0	5.0	8.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3	5	8	

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	?/ /
REVIEW & APPROVAL	/ /	PROFESSIONAL & TECHNICAL ASSISTANCE	/ /
BIOLOGIC STANDARDS	/ /		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

The Sanitary Engineering Branch, DRS, provides some space, equipment, and engineering assistance for Germ-free Animal Studies project.

For Studies on Amoebiasis, LOBUND, University of Notre Dame, South Bend, Indiana, provides facilities.

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-74
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Helminthic Diseases
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of Helminths and the Diseases Caused by Them
PROJECT TITLE

7. Dr. Louis J. Olivier
PRINCIPAL INVESTIGATOR

8. Dr. E.G. Berry, Dr. E.B. Cram, Dr. W.B. DeWitt, Dr. W.T. Haskins, Dr. M.F. Jones, Mrs. M.O. Nolan, Dr. Kenneth Warren, Dr. P.F. Weinstein
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

Project subtitle:

Host-parasite Relations in Worm Infections of Laboratory Animals

Objectives:

To study the characteristics of infection in laboratory animals; to learn how resistance to infection may be enhanced and reduced, to learn how the host defense mechanisms act to resist infection; and to study variations in resistance in host strains.

Methods Employed:

Various quantitative infection techniques including transplantation of the parasites to new hosts and to new locations in the host. Techniques to measure and to transfer resistance. Study of resistance to initial infection and to superinfection. Study of the effects of modification of the hormone balance on susceptibility to infection.

Further information has also been obtained concerning vitamin requirements of the free-living stages and a standardized culture medium has been developed which may allow such studies as the testing of drugs, antibody evaluation, etc., on larval growth.

Significance to the Program of the Institute:

When the nutritional and physical conditions necessary for development of nematodes, and the identity of excretory products are known, basic in vitro studies may explain clinical and pathological effects of nematode infections and enable better diagnosis and treatment.

Proposed Course of Project:

Work will be concerned with the investigation of different tissue and blood fractions to delineate further the growth requirements of nematodes in complex media, and attempts will be made to obtain development in media of known chemical composition. A further study will be made of the nitrogenous materials found in the larval incubates and to gain quantitative information concerning protein and nonprotein excretory products.

Project Subtitle:

Investigation of Intermediate Hosts and Vectors of Human Diseases Caused by Worms

Objectives:

To learn the distribution, life history and ecology of the intermediate hosts and to develop a rational and useful system of classification and means of identification. This knowledge is essential if one is to understand the epidemiology of worm infections spread by vectors and if one is to devise effective vector control measures. To devise means of control of the vectors since this is often the most effective way to control the disease which they transmit.

Methods Employed:

Colonies of known vectors are maintained in the laboratory where they are available for intensive study. Suspected vectors are exposed to infection to evaluate their suitability as vectors in the field. Attempts are made to discover means for controlling the vectors in the field by subjecting them to poisons, etc.

Major Findings:

Progress has been made toward the discovery of criteria useful for the accurate identification of the snails which serve as the intermediate hosts of Schistosoma mansoni and Schistosoma haematobium.

Significance to the Program of the Institute:

At the present time the knowledge of the distribution, biology,

ecology and taxonomy of the intermediate hosts of human schistosomes is very limited and adequate understanding of the epidemiology and control of schistosomiasis awaits progress in this field.

Proposed Course of Project:

Continue anatomical studies of snails in order to make possible their accurate identification. Study variation among snail strains with respect to susceptibility to infection with schistosomes.

Project Subtitle:

An Investigation of Pneumocystis as an Etiologic Agent in Human Cases of Atypical Pneumonia

Objectives:

A study of the biology of Pneumocystis especially to elucidate the nature of the parasite, its life cycle, and method of transmission. This is necessary for prosecution of studies leading toward an investigation of the role of Pneumocystis in human cases of atypical interstitial pneumonia.

Methods Employed:

Presence of the organism is determined by microscopic examination of Giemsa-stained smears or impressions of lung tissues. Techniques for isolation and concentration of the pneumocysts need added study. Inoculation of material from infected rats in attempt to transmit infection. In vitro culture procedures may be attempted.

Major Findings:

Occurrence of the organisms in white rats over 8 weeks of age has been confirmed. The highest incidence is in rats 11 to 13 weeks old. Attempts to transmit the organism from rat to rat or to other laboratory animals via subcutaneous, intraperitoneal, and intranasal routes have been unsuccessful. It has been shown that the multiplication and growth of the organisms in infected rats is not affected by administration of cortisone acetate.

Significance to the Program of the Institute:

Fatal cases of Pneumocystis pneumonia, hitherto not known to exist in the United States, have been reported from Connecticut, Illinois, and Oklahoma. The disease may be more prevalent in the United States than medical records indicate.

Proposed Course of Project:

It is planned to continue laboratory studies on the nature, life cycle, transmission, and culture of the agent. The pathology of the infection will be studied and search will be made for further evidence of the existence of Pneumocystis in the human population.

Project Subtitle:

Nutritional Aspects of Helminthiasis

Objectives:

To determine the role of nutritional factors in the disease processes associated with helminthic infections.

Methods Employed:

Experimental animals maintained on defined diets deficient in one or more factors are exposed to various helminths. Influence of the induced deficiency on the host as well as on the parasites and its relations with the host are evaluated by biological, biochemical, pathological, and physical methods. Special attention is given to the influence of the deficient state on disease patterns associated with the infection.

Major Findings:

A mouse diet deficient in Vitamin E, cystine, and Factor 3 produced multiple necrotic degeneration of liver, heart, pancreas, kidney, and heart muscle. When mice so affected were exposed to Schistosoma mansoni infection they acquired a greater worm burden than normal controls but the worms failed to mature. This indicates that the deficient mice had greater susceptibility to infection but failed to provide a suitable milieu for development of the worms.

Significance to the Program of the Institute:

It has become quite clear that debilitating helminthiases occur very frequently among populations with dietary deficiencies. The disease state of these individuals cannot be properly evaluated and dealt with until the interaction of these factors is better understood. The problems of treatment, prevention, and control of helminth infections can be met more effectively if these relations are understood.

Proposed Course of Project:

The study of deficient diets and their relation to worm infections will continue. The Schistosoma mansoni infections will be studied in mice with fatty liver cirrhosis produced by deficient diet. Later the study will extend to the relations between diet and other worm infections.

Project Subtitle:

Pathological Physiology of Schistosome Infections

Objectives:

Study the pathology of schistosome infections with special reference to portal collateral circulation, including esophageal varices. Determine whether the brain becomes more sensitive to toxins implicated in hepatic coma (ammonium compounds) when cirrhosis with portal col-

lateral circulation exists.

Methods Employed:

Dissection of animals at intervals following infection with Schistosoma mansoni to study development of collateral circulation. Intravenous injection of ammonium compounds into normal animals and into those with induced liver damage and portal collateral circulation, and observation of effects.

Major Findings:

Esophageal Varices begin to develop in mice about 11 or 12 weeks following exposure to infection with Schistosoma mansoni. Intravenous ammonium bicarbonate appears to be one-third more toxic, and ammonium citrate five times more toxic, than ammonium chloride. The greater toxicity of the first compound may be due to the high pH induced while the increased toxicity of the second compound may be due to induced hypocalcemia.

Significance to the Program of the Institute:

Mice infected with Schistosoma mansoni are good tools for the study of schistosome pathology since they are readily infected and develop symptoms rapidly. They are useful for the study of broad problems involving the circulatory system since they develop portal collateral circulation as a result of the infection.

Proposed Course of Project:

Continue the study of the toxicity of ammonium compounds in normal and infected animals. Study the relation of cirrhosis and portal collateral circulation to the action of these compounds. Investigate the occurrence of hepatic coma in advanced cases of human schistosomiasis.

Project Subtitle:

Role of Certain Helminths in the Causation of Cancer

Objectives:

Clinical observations and statistical surveys have suggested that some of the worm parasites of man may be related to the development of malignant growths. One objective will be to test whether such a relation can be demonstrated in the laboratory. Taenia taeniaformis is known to cause liver tumors in rats. Attempts will be made to learn how and why the tumors occur.

Methods Employed:

Schistosoma haematobium. Large numbers of hamsters are being infected with this species and held for long periods of time in an attempt to learn whether the infection gives rise to malignant growths in the bladder or other organs. Two strains of the parasite are being used since there

is some field and laboratory evidence that they may differ in virulence and therefore, by inference, in their tendency to induce tumors.

Taenia taeniaformis. This tapeworm regularly produces liver tumors in rats. Attempts are being made tolearn whether a virus may be involved by using virus isolation and passage techniques.

Major Findings:

The studies have not progressed far enough to produce reportable results. The host tissue specimens obtained from the S. haematobium study should produce valuable incidental data on the pathology of infection with this species.

Significance to the Program of the Institute:

Since worms often produce chronic infection in man they have long been suspected of contributing to the development of malignant growths. Study of the relation of the worms to malignancy should not only increase our knowledge of the pathogenicity of the worms but give useful new information concerning the factors that predispose the individual to cancer.

Proposed Course of Project:

The course the S. haematobium study will take depends upon whether the infection can be shown to be related to the development of cancer. Other worm infection may be studied in the same way. In the T. taeniaformis study, the emphasis will be on the causation of the tumor growth.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-74
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$101,317	$35,500	$136,817	9.0	6.0	15.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	9	6	15	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Pathological Anatomy Branch, NCI, provides animal space and care and makes pathological studies involving Schistosoma haematobium for the project on the Role of Certain Helminths in the Causation of Cancer.

Cancer Products Unit, DBS, cooperates in the study of the relation of the larvae of Taenia taeniaformis to cancer production for the above-mentioned project.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications

15. NIAID-74
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Cram, E. B.: Stepping stones in the history of the American Society of Parasitologists. J. Parasitol., 42, 461-473, October 1956

Weinbach, E. C. and Nolan, M. O.: The effect of pentachlorophenol on the metabolism of the snail Australorbis glabratus. Exper. Parasitol., 5, 276-284, May 1956.

Weinstein, P. P. and Jones, M. F. : The in vitro cultivation of Nippostrongylus muris to the adult stage. J. Parasitol., 42, 215-236, June 1956.

In Press

Haskins, W. T. and Weinstein, P. P.: Amino acids excreted by Trichinella spiralis larvae. J. Parasitol.

Haskins, W. T. and Weinstein, P. P.: The amine constituents from the excretory products of Ascaris lumbricoides and Trichinella spiralis larvae. J. Parasitol.

Haskins, W. T. and Weinstein, P. P.: Nitrogenous excretory products of Trichinella spiralis larvae. J. Parasitol.

Nolan, M. O. and Wright, W. H.: Preliminary studies on Pneumocystis carnii. J. Parasitol.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Dr. W. B. DeWitt was elected to the Society of Sigma Xi, George Washington University Chapter.

Dr. E. G. Berry was appointed Honorary Curator of the Museum of Zoology, University of Michigan.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-75
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Medical Entomology
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies of Arthropods, Their Control and Virus-Arthropod Relations
PROJECT TITLE

7. Dr. Paul A. Woke
PRINCIPAL INVESTIGATOR

8. Mr. H. T. Dalmat, Dr. J. C. Jones
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

Project Subtitle:

Pathogen-arthropod Host Relations with Special Reference to Survival and Multiplication of Viruses in Arthropod Tissues.

Objectives:

To develop nutrient media suitable for the maintenance of arthropod organs and tissues as a basis upon which to investigate the possibilities of survival and multiplication of viruses in such cultures as well as in living arthropods.

Methods Employed:

Organs and tissues are incubated in culture media of varied formulations to find the most suitable for maintaining survival and normal activities of organs and tissues. Virus is inoculated into the hemocoele, incubated for 3 and 6 days at 28° C., and titrated in culture media.

Major Findings:

Virus of human poliomyelitis was recovered from one of nine lots of southern armyworm pupae into which the virus had been injected six days earlier. This lot was age 2 to 3 days at the time of inoculation. Moths in southern armyworm pupae that were inoculated with human poliomyelitis virus continued development to maturity, but 4 of 5 were defective.

Ovaries of Aedes aegypti survived for over 80 hours and guts survived for 67 hours in an aerated saline formulation of Bradford and Ramsey (1949) modified to include a small amount of magnesium and glucose. Tissues of Aedes aegypti survived in this solution supplemented with extract of Supella supellectinium whole nymphs for 11 to 16 days. High magnesium content of the medium was shown to be distinctly harmful to the survival of organs, whereas the addition of horse serum was followed by marked improvement. Tissues survived for periods up to 4 and 5 weeks in a partially defined medium modified from one used for cultivation of Strain L cells.

In this medium in hanging drop, elongate, rounded, and spindle shaped cells appeared on the coverslips and apparently increased in numbers for 4 or 5 days, from various tissues of the egg, larva, pupa, and adult of Aedes aegypti.

Information thought to be needed for further tissue culture and virus research investigations, on the gross anatomy and development of the ovaries of Aedes aegypti was ascertained.

Significance to the Program of the Institute:

These findings may be useful in the development of media for the maintenance of arthropod organs and culture of cells and tissues. Achievement of this goal will provide the means for advance in understanding of arthropod-borne pathogens and pathogen-arthropod host interrelations. Studies can then be made on the possible cultivation of viruses such as that of poliomyelitis in arthropod tissue culture, identification of the specific tissues that support virus multiplication, determination of potential vectors in tissue culture, and virus-arthropod tissue relations.

Proposed Course of Project:

Discontinued September 28, 1956.

Project Subtitle:

Ecology and Behavior of Arthropods of Public Health Importance

Objectives:

To learn about the interrelations of arthropods and their environment as a basis for further studies in disease transmission and control.

Methods Employed:

Reactions of the arthropod are correlated with the measured environmental forces and stresses that act as stimuli to activity in the natural habitat and in the laboratory.

Major Findings:

A long-term basic research program to study the ecology and behavior of the vectors of tropical arthropod-borne viral diseases was authorized on September 28, 1956. A preliminary field investigation of dry season conditions in Central America was commenced on November 14.

Significance to the Program of the Institute:

Since tropical arthropod-borne diseases continue to have great worldwide military and civil importance, and since these diseases threaten residents of the United States of America and United States citizens abroad, the circumstances of their transmission must be made known and means for control of the diseases and of the vectors must be found. Knowledge gained from a study of the ecology and behavior of the vectors is of the greatest importance as a basis for further studies in transmission and for the development of control procedures.

Proposed Course of Project:

The problem of tropical arthropod-borne diseases will be approached by a long-term basic research program to obtain a thorough understanding of the vectors. The unique problem presented by the vast forests and unusual habitats and behavior of known vectors demands new approaches in control which can be developed only on the basis of new fundamental knowledge.

Project Subtitle:

Arthropod Transmission of Virus Neoplasms of Cottontail Rabbits (Shope's Fibroma and Shope's Papilloma)

Objectives:

To determine (1) the mechanism of transmission of Shope's virus fibroma and papilloma of cottontails; (2) relation of tumor histology to virus transmission; (3) incidence of infection in endemic areas; and (4) duration of immunity.

Methods Employed:

Arthropods are permitted to feed on tumors of different ages and induced by different techniques. The arthropods either feed interruptedly on the tumor and then on a normal host, or they are held after engorgement

on tumors for varying periods of time. Their mouthparts may be ground up and prepared in suspensions which are either inoculated in tenfold dilutions, or may be abraded into the skin. Tumors are harvested from animals and are preserved in 0.5 gm. quantities in beef infusion broth by freezing. Titrations of tumor suspensions or of suspensions of arthropod mouthparts are made by serial intracutaneous inoculations into rabbits. Carcinogens were applied to animals topically while cortisone was given subcutaneously. Rabbits were exposed to X-rays by use of a 200 KV Westinghouse X-ray machine with dual tubes. All neutralization tests were done in vivo and 100 infectious doses of virus were used with twofold dilutions of sera being tested.

Major Findings:

Fibroma: Aedes aegypti, Anopheles quadrimaculatus, Triatoma infestans, Rhodnius prolixus, and Cimex lectularius are all capable of transmitting fibroma both by interrupted feed, as well as by a second feed after a delay from the infective meal. Although it was found that virus did not proliferate in the arthropod vectors, it is viable in their mouthparts for long periods of time. In living mosquitoes transmission was possible after 35 days and a suspension of ground mouthparts of infected mosquitoes was still virulent 18 days after preparation of the suspension, when kept at room temperature. Virus is localized primarily in the stylets and clypeal region with very little to none in the head capsule. Virus found from time to time in the abdominal region appears to be accidental -- either due to virus particles ingested with blood meal, or virus that breaks loose from its lodging in the mouthparts and passes to the intestinal tract.

At the peak of tumor development in the cottontail rabbit (15 to 25 days) and in the domestic rabbit (10 to 12 days), the virus titer is equivalent from similar inocula. However, arthropods usually can transmit from the cottontail tumors but not from those of domestic animals. Sucking domestic rabbits, on the other hand, will be infective to mosquitoes from about 7 days on. When domestic rabbits are given total body irradiation (350 r) prior to being infected, the tumors will last longer and will be infective to arthropods. Although X-rays will affect presumably the immunity reaction of the rabbits, carcinogens and cortisone did not seem to give similar results.

It was found that although animals that have recovered from fibroma infection (tumors regressed) are refractive to further infection with this virus, they maintain neutralizing antibodies for only about 3 to 4 months.

Up to the present, fibroma virus could be propagated only in rabbits and on the chorioallantoic membrane of chicken eggs. However, successful passages have been achieved from rabbit to brain of one-day-old mice and back to rabbit. The mouse strain is being continued with the hope that it can be maintained.

Papilloma: It has been possible to transmit papillomas from cottontail to cottontail by both interrupted feed and by delayed feeding of Aedes aegypti, Anopheles quadrimaculatus, and Rhodnius prolixus. This represents the first successful transmission by insects reported to date. There is one record of transmission by the rabbit tick, Haemaphysalis leporispalustris, but this was possible only by interrupted meal. We have also been able to transmit by insects from what appear to be metastatic lesions of carcinomas derived from the papillomas (as checked by cancer investigator), but the tumors are still in the process of being studied histologically. The tumors so transmitted have been passed from domestic to domestic rabbit and superficially do not resemble papillomas. Should the tumors prove to be carcinomas, this will mean that virus has for the first time been extracted from the malignancies derived from papillomas and from the tumors of domestic rabbit (formerly considered to be masked), thereby lending weight to the theory that viruses may be causative agents of cancer.

Significance to the Program of the Institute:

With our present concern over cancer, it seems desirable to work on neoplasms other than human ones, to determine the mechanism by which they are initiated and grown. Two such diseases are Shope's cottontail fibroma, which is not a true cancer since it usually regresses, and Shope's cottontail papilloma, which has importance since it often develops into carcinomas.

Proposed Course of Project:

This program probably will be brought to an end. More work will be done on the mouse-adapted strain and on the matter of the possibility of tissue immunity without humoral antibody.

Project Subtitle:

Studies on the Mode of Action of Insecticides with Special Reference to the Nature of Tolerance and the Development of Resistance.

Objectives:

(1) To define levels of DDT tolerance in Anopheles larvae and pupae and attempt to raise or lower these levels. (2) To attempt to apply the exposure testing method to dieldrin.

Methods Employed:

Expose larvae and pupae of known ages and sexes to freshly prepared acetone suspensions of DDT or dieldrin for 1 minute to 6 hours, wash animals with water and observe mortality rates.

Major Findings:

(1) Different levels of tolerance to DDT were defined with precision for third and fourth stage larvae and pupae, revealing a 9- to 12-fold normal increase in tolerance during the last larval stage alone, and further showing that pupae are normally 48 to 58 times more tolerant of DDT than 1-hour-old fourth instars.

(2) In the larval stages both sexes are equally tolerant, but in the pupal stage the females are significantly more tolerant than males.

(3) Feeding and overcrowding of larvae during exposure to DDT do not significantly alter the median lethal time of exposure, but 24-hour starvation of larvae greatly reduces their natural tolerance.

(4) With dieldrin, the exposure testing technique does not give accurate or rapid estimations of tolerance in 24 hours because this insecticide tends to have a remarkable delayed effect. For example, after a 5-minute exposure of 24-hour-old pupae to dieldrin 1:200,000, the pupae are able to emerge as adults but die usually on the day of emergence.

(5) A most important finding now under active study is that ether anesthesia temporarily prevents the attachment of the DDT molecule during exposure testing.

Significance to the Program of the Institute:

More rapid and accurate laboratory and field estimations of DDT tolerance in larval Anopheles mosquitoes made possible by the exposure testing method. A method for estimating tolerance in pupae was devised where none existed heretofore.

The discovery of the delayed effects with dieldrin exposures suggests that tests now being used in other laboratories may be partially invalidated.

The discovery that during deep anesthesia larvae are protected from the action of DDT has fundamental significance in studies on the still unknown mode of action of DDT.

Proposed Course of Project:

To continue current studies with DDT and dieldrin, with special regard to the effects of different anesthetics on the action of DDT, and to the establishment of a more valid standard for detecting and measuring tolerance to dieldrin.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-75
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$45,572	16,000	61,572	3.0	4.0	7.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	4	4	8	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

For the project Arthropod Transmission of Virus Neoplasms of Cottontail Rabbits (Shope's Fibroma and Shope's Papilloma), NCI cooperates by doing electron microscope studies, preparing histological material, and, when requested, irradiating animals. Missouri Conservation Department supplies wild cottontail rabbits and the Wildlife Pathology Section of the Patuxent Research Refuge supplies naturally-injected cottontails (fibroma).

(Use reverse and additional pages, if necessary)

Calendar Year 1956

PUBLIC HEALTH SERVICE - -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-75
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Jones, J. C.: A portable adult mosquito feeding unit. Mosq. News, 16, 230-231, September 1956.

Jones, J. C.: An improved guinea pig restrainer. Mosq. News, 16, 149, June 1956.

Jones, J. C.: A study of normal heart rates in intact Anopheles quadrimaculatus Say larvae. J. Exper. Zool., 131, 223-233, March 1956.

Jones, J. C.: Effects of different gas concentrations on heart rates of intact Anopheles quadrimaculatus Say larvae. J. Exper. Zool., 131, 257-265, April 1956.

Komp, W. H. W.: Notes on mosquitoes from an area of endemic yellow fever in Colombia. Proc. Entom. Soc. Wash., 58, 37-42, February 1956.

Woke, P. A.; Stone, A.; and Foote, R. H.: Obituary: William H. Wood Komp, 1893-1955. Proc. Entom. Soc. Wash., 58, 47-55, February 1956.

In Press

Jones, J. C.: A simple method for sexing living Anopheles larvae. Ann. Entom. Soc. Am.

Jones, J. C.: Effect of various poisons on Anopheles heart rates. J. Exper. Zool.

Jones, J. C.: Total and differential hemocyte counts in mealworms poisoned with DDT. J. Cell. & Comp. Physiol.

Jones, J. C.: Methanol blackening of Sarcophaga cuticles. J. Exper. Zool.

Jones, J. C.: Effects of salts on Anopheles quadrimaculatus Say with special reference to the heart rate. J. Exper. Zool.

Jones, J. C.: A new standard for the rapid detection of DDT tolerance in Anopheles quadrimaculatus larvae and pupae. Mosq. News.

Jones, J. C.: The hemocytes of Sarcophaga bullata Parker. J. Morphol.

Dalmat, H. T.: Biology and control of simuliid (Diptera) vectors of onchocerciasis in Central America. Proc. Tenth Intl Cong. Entom. Canada.

Woke, P. A. and Rosenberger, C. R., Jr.: Survival of mouse poliomyelitis virus in living invertebrates. J. Infec. Dis.

Jones, J. C.: Effects of drugs on Anopheles heart rates. J. Exper. Zool.

Jones, J. C.: Heart rates in insects. (Table) Handbook Biol. Data.

Jones, J. C.: Effects of various physiological conditions on circulation in insects. (Tables) Handbook Biol. Data

Dalmat, H. T.: Is Simulium dinelli Joan (1912) (Diptera, Simuliidae) present in Guatemala? Ann. Entom. Soc. Am.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

Individual Project Report

Part A. Project Description Sheet

1. NIAID-76
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Epidemiology
SECTION OR SERVICE

5. Memphis, Tennessee and Columbia, South Carolina
LOCATION (IF OTHER THAN BETHESDA)

6. Epidemiology of Parasitic Diseases
PROJECT TITLE

7. Dr. Martin D. Young
PRINCIPAL INVESTIGATOR

8. Dr. D. E. Eyles, Dr. C. L. Gibson, Dr. R. W. Burgess, Dr. G. D. Wolcott, Dr. G. M. Jeffery
OTHER INVESTIGATORS

10. PROJECT DESCRIPTION

Project Subtitle:

Virus-Parasite Associations.

Objectives:

To determine experimentally if parasites might be associated with some of the virus diseases of man or animals as vectors or in some other capacity. If experimental evidence establishes such relationships between parasites and viruses, further objectives are to study such relationships under natural conditions and to investigate known virus infections and known populations to determine existing relationships. This includes the study of possible intestinal viruses among institutionalized mental patients. To investigate the possibility that certain parasites may themselves be afflicted with harmful virus infections. To investigate the effects of parasitic infections upon virus infections and vice versa.

Methods Employed:

Small animals and their common parasites are used in attempts at transmission of viruses. Viruses, such as LCM, polio, and equine encephalitis, are maintained in small animals by serial transmission, providing infective virus material for experimental work. Viruses and parasites are brought together in various ways, such as by simultaneous infection in animals, during cultivation of the free-living stages, or by simultaneous exposure of the host to infective parasite stages and virus suspensions. Tissue culture or small animals are used for detection of virus infection carried by parasites from virus-infected animals, for virus detection in parasites suspected of harboring viruses in nature, and for detection of virus infection in arthropods. Incidence of intestinal viruses and their possible association with parasites in institutionalized mental patients are studied with animal inoculation, tissue culture, and other means.

Major Findings:

The initial phase of the experimental work on the mechanical transmission of rabbit viruses was completed and the results showed conclusively that papilloma virus can be mechanically transmitted to rabbits with the penetrating larvae of Nippostrongylus muris. Such has not been the case when the fibroma virus was similarly tested. Also the papilloma virus was successfully transmitted in one experiment where the larvae of human hookworm were used as mechanical carriers and in a single trial using the larvae of Ancylostoma caninum. Efforts to introduce the rabbit viruses into the helminth larvae by exposure of the larvae to virus material during the free-living stages have been unsuccessful in several preliminary experiments.

Studies of association of Nippostrongylus muris and LCM virus have served to confirm the prior failure to transmit virus from mouse to mouse with parasite-derived material. Results of experiments designed to incorporate virus into this parasite during larval development in culture are not yet conclusive.

Significance to the Program of the Institute:

Many problems associated with the transmission of viruses in human and animal populations remain unsolved. New viruses in mammals and arthropods are continually being discovered. There are suggestions in the literature and theoretical possibilities that parasites may act as reservoirs and agents of transmission for viruses of importance to man. Elucidation of association of viruses and parasites, both experimental and natural, will do much toward bringing about a more complete understanding of the epidemiology of diseases produced by such organisms, alone or in combination.

Proposed Course of Project:

Axenic cultivation of nematode larvae will afford new opportunity for exposing the parasites to virus infection during development and for detecting possible incorporation of virus. Tissue culture methods will be used to a greater extent for detection of virus in suspect material. There will be a trend toward using viral agents known to be normally found in intestinal environment, such as the Lansing strain of polio virus now in use. Other than the substitution of the newer methods and new virus-host-parasite combinations, the general course of the project will be changed very little. The use of available human material will be emphasized when appropriate material becomes available.

Project Subtitle:

Virus-Arthropod Relationships.

Objectives:

To investigate possible relationships between various arthropods and viruses including the maintenance of infective virus through immature stages of insects following acquisition by ingestion or other means and investigation of viruses of arthropods and their medical importance.

Methods Employed:

Materials from virus-infected mice are introduced into pans of larvae of mosquitoes. Subsequently, larvae and adult mosquitoes are tested for presence of virus.

Major Findings:

Preliminary experiments completed during the year demonstrated the probable incorporation of LCM virus into the larvae of anopheline mosquitoes and the maintenance of viable virus through subsequent larval and adult stages.

Significance to the Program of the Institute:

The possibility that virus infections may be acquired by arthropods in the larval stage and carried or multiplied in a subsequent stage is of importance. Elucidation of these and other virus-arthropod associations will add to the epidemiologic knowledge of these diseases.

Proposed Course of Project:

An extension of the work involving different viruses, arthropods and vertebrate hosts is under way. Arthropods differing from mosquitoes, such as eye-gnats, will be tried.

Project Subtitle:

Epidemiology of Intestinal Parasites in Institutional Populations.

Objectives:

To investigate factors governing the incidence and spread of intestinal parasites in institutional populations. Wherever possible, knowledge gained from work within the institution is applied to more normal populations in an effort to provide answers to some of the existing epidemiological problems.

Methods Employed:

Many epidemiological approaches are used to elucidate differential transmission of parasites, including examinations of several types of institutionalized patients, examination of premises for contamination, laboratory studies of parasite species, and other methods. Parasite incidences are examined for relationship to certain mental and physical conditions in the host and in regard to other existing conditions in the host and in the environment.

Major Findings:

Completion of two years of examinations of a closely controlled population in a single building has yielded some interesting data and conclusions. The parasite incidences have remained quite stable during the period, although there has been much gain and loss of parasites by individual patients. There is evidence of a continued high rate of transmission of protozoan parasites in spite of the generally high level of sanitation in the environment. The new building has yielded some evidence of contamination but this has not built up to any large degree at the end of the observation period. There has been an apparent cessation of hookworm and Trichuris transmission, although the rates of infection with these parasites have remained high because of long persistence.

Studies on the rare parasite Isospora belli were continued through the early part of the year and were reopened on discovery of an epidemic of this parasite in the patients and personnel of the school for mental defectives during the last quarter. Laboratory studies indicated that the parasite is not infective to mice, rats, guinea pigs, swine, rabbits, dogs or rhesus monkeys. Clinical records collected on a number of infections in normal individuals have clarified the clinical course of infection. Investigations of possible epidemiological factors involved in the spread of this parasite have been done; only gross contamination of the environment can be incriminated to date.

Significance to the Program of the Institute:

To understand more clearly the factors affecting the spread of parasitism in normal populations, much can be learned utilizing the controlled population within an institution. The higher incidence of parasitism in the institutional group makes it an ideal group for study of these parasites and their epidemiology. Much should be learned about the relationship of parasite spread to housing, age, length of residence, and other variables.

Proposed Course of Project:

The epidemiological study of parasites in the institutional population completed for a two-year period will be continued during the ensuing year with examinations of the group at the less frequent intervals of six months. The emphasis will continue to be on the rate of transmission of parasites, on the possible increased rate

of loss of those parasites with a low transmission rate, and on observation of the contamination level in the building. Biological studies on parasites whenever unusual material becomes available will be done to elucidate the epidemiology of the parasites; this will apply particularly to the parasites found less commonly in normal populations. Further steps will be taken during the year to correlate such things as age, physical or mental disability, and other host factors with parasite incidences.

Project Subtitle:

Chemotherapy of Institutional Parasitoses.

Objectives:

To find adequate drugs for the prophylaxis and the treatment of parasitic infections, and especially hookworm, Trichuris and Strongyloides, which constitute serious health problems in certain types of mental patients and in some more normal population.

Methods Employed:

Promising compounds in varying dosages are tried against parasitic infections.

Major Findings:

Some of the parasites which constitute serious health problems in institutions, and in some normal populations, are those for which there is no satisfactory treatment. Several compounds tested recently showed encouraging results. Toluene removes more hookworms than any other drug tried. Puromycin showed encouraging activity against Trichuris. Piperazine also removed the above two parasites but at lesser rates. Puromycin acted both suppressively and prophylactically against gut protozoa under conditions of very high exposure risks.

Significance to the Program of the Institute:

It is evident that mental hospitals are important reservoirs of parasitic infections and many of the patients are adversely affected by the parasites. In spite of advances in sanitation, there are still normal population areas which have clinical

parasitosis. The development of drugs to reduce parasitism is important to the health of the mental patient as well as to others. The finding of good prophylactic effects of puromycin indicates such use in areas of high exposures not only in hospitals but also under other conditions.

Proposed Course of Project:

Trials of new drugs will continue. Special emphasis will be placed on enhancing the effects of active drugs by combining them for possible synergistic effect; progress in this effort will depend upon preliminary testing of combined drugs in lower animals.

Project Subtitle:

Cytological Studies of Parasitic Protozoa.

Objectives:

To study chromosome number, morphology, and behavior in parasitic protozoa, with emphasis on the human blood protozoa. To locate where possible the point in the life cycle at which meiosis occurs in order to lay a basis for possible genetic studies.

Methods Employed:

The nuclei of parasitic protozoa, mostly human, are studied in fixed and stained preparations and in the living condition under bright field and phase contrast microscopes. The parasites are studied at all possible stages of their life cycles, under normal and experimental conditions.

Major Findings:

Observations on chromosome number in Plasmodium species indicate that meiosis takes place just after the ookinete rounds up to form an oocyst on the mosquito gut. Contrary to reports in the literature the nuclei of the gametocytes of Plasmodium falciparum were shown to contain desoxyribonucleic acid by the Feulgen test. The nucleus in Plasmodium species was found to exhibit an apparently autonomous movement, independent of the cytoplasm. When Trypanosoma gambiense in rats was treated with puromycin several cytoplasmic effects were found, including the formation of two distinctive types of inclusion bodies. Nuclear division was inhibited.

Significance to the Program of the Institute:

There is little basic knowledge of nuclear structure and behavior in most human protozoan parasites. Information of this sort will aid in fields such as taxonomy, and will make possible an understanding of the inheritance of such phenomena as virulence and drug fastness, as well as of morphological strain and species differences, in these parasites.

Proposed Course of Project:

(a) Study material on hand in order to analyze meiosis in Plasmodium; (b) Obtain photographic documentation of nuclear movement in Plasmodium and of the effect of puromycin on trypanosomes; (c) Continue efforts to analyze African trypanosomes cytologically. This last is difficult but it is felt that the possible ultimate value of such information makes a continuation of this study worthwhile.

Project Subtitle:

Cytogenetics and Species Relations of Insect Disease Vectors.

Objectives:

Correlation between chromosomal patterns and inheritance of morphological and physiological characters in species, populations, hybrids and selected breeding lines of mosquitoes and other arthropods. Determine validity of and relationships between species.

Methods Employed:

Rear and study characters of species, variants of species, hybrids, selected strains. Make cytological studies and chromosome maps of as many as possible for study and comparison.

Major Findings:

A study of the salivary gland chromosomes of Anopheles freeborni showed the band pattern in chromosome I to be quite unlike that of A. quadrimaculatus previously reported. The salivary gland chromosomes of fourth stage larvae of hybrids between A. aztecus and A. freeborni have many unpaired segments indicating that though the species will cross the gene arrangement in the chromosomes is quite different in these species.

Significance to the Program of the Institute:

Ability of arthropods to transmit disease and susceptibility to insecticides as well as other physiological and morphological characters may depend upon specific characters, inheritance of which may be demonstrated by a combination of breeding, chromosomal, and taxonomic studies.

Proposed Course of Project:

This project will not be continued because of a shift in emphasis to other studies.

Project Subtitle:

Genetic Selection in Anopheline Mosquitoes.

Objectives:

To elucidate inheritance of certain morphological characters by selective breeding.

Methods Employed:

Anopheles freeborni only rarely has the antepalmate hairs in an unbranched (single) state on the fourth and fifth abdominal segments of the larvae. These hairs are most often double or triple but may have as many as seven branches. Adults reared from larvae showing the unbranched hairs were selectively bred through sixteen generations and other larvae having double branches were selectively bred for eleven generations.

Major Findings:

The single hair line showed a sudden increase in this characteristic in the first three or four generations but little further change thereafter. The double branch line showed little tendency toward stabilizing the proportion of this characteristic produced. Certain other characteristics, probably mutations, appeared such as airplane wings on adults and unicorn inner clypeal hairs on fourth stage larvae.

Significance to the Program of the Institute:

The selective breeding for hair characteristics indicates that these characters are probably of a multiple gene type. It also demonstrated that the characteristics of one species, i.e., multiple hair branches in A. freeborni, could be selectively bred to have the characteristics of another species, i.e., predominantly single hairs of A. quadrimaculatus. It is possible that ability to transmit disease could be also selectively bred.

Proposed Course of Project:

The project has been ended because of emphasis on other projects.

Project Subtitle:

Malaria Studies.

Objectives:

To add to the biological knowledge of malaria by limited observations on induced malaria and experimental studies. To serve as probably the only source in the nation for established strains of malaria for use on a nationwide basis for the therapy of neurosyphilis, nephrosis, Parkinsonism, and other conditions and for experimental malaria studies.

Methods Employed:

Malaria is induced in neurosyphilitic patients and in volunteers under normal and experimental conditions. During the course of the infection, pertinent biological information is obtained. Malaria is being furnished to several institutions to treat nephrosis and for experimental work.

Major Findings:

Patients who had experienced P. vivax infections as long as 13 years before being challenged by a subsequent infection still showed an immunity which was manifested in varying ways, some of which have epidemiological significance. The subsequent infections often showed an abortive primary course in that after several fevers the parasite density would drop suddenly

as if an antimalarial drug had been given. These infections did not infect mosquitoes so well as did the original infections of the same species. There was some indication that the immunity was species, rather than strain, specific.

The response of P. malariae infections to pyrimethamine (Daraprim) was found to be very slow and relapses occurred, contrary to the prevailing opinion that plasmodia resistance to pyrimethamine occurs only when subcurative doses were given.

Significance to the Program of the Institute:

The persistence of vivax malaria immunity over many years and the ameliorating effects upon subsequent infections is significant epidemiologically. It indicates that in subsequent infections, even many years later, the patient will be sick for a shorter period of time and that he will be less likely to serve as a focus for infection. This emphasizes that in endemic malarious areas the primary attack, usually in children, is the most important factor in illness and in the spread of the disease.

Proposed Course of Project:

It will operate along the presently curtailed lines with the principal activity being that as a center for furnishing inocula for the treating of neurosyphilis, nephrosis, and other diseases. Data collected from years of experimental work is being organized for reports.

Project Subtitle:

Biological Studies of Toxoplasma gondii.

Objectives:

To investigate parasitological problems involving Toxoplasma particularly those arising from epidemiological and chemotherapeutic studies, and to make fundamental studies of the life cycle of the parasite in animals and in man.

Methods Employed:

Toxoplasma infections are induced by various methods in animals which may serve as natural reservoirs of the infection. Those features of the infection which might have bearing on the epidemiology of the disease are particularly observed, such as the occurrence of organisms in secretions and excretions, the persistence of parasites in tissues, and the resistance of these parasites to physical and chemical conditions. Serological studies are coordinated with the parasitological studies in order to gain insight into the specificity of serological procedures. Studies are being made of changes of virulence of strains of Toxoplasma and the effects of corticosteroids upon virulence. Other experiments are being conducted to study the effect of other parasite infections on Toxoplasma serological findings.

Major Findings:

Studies on naturally infected hosts, particularly cats, have disclosed a high degree of correlation between parasitologic findings and serologic findings using the dye test for toxoplasmosis. Parasites were nearly always recovered from cats with high positive tests (1:256 and higher) and over half the time from cats with titers of 1:64. These data provide good evidence of the specificity of the test. Toxoplasma were recovered from the brain of a patient dying of cancer, but who had been suspected of suffering from toxoplasmic chorioretinitis. Studies were made of the adaptation of newly isolated strains to the laboratory mouse. Gradual changes in virulence as passage continued were detected. The use of cortisone apparently did not increase the rapidity of adaptation. A comparison of methods of the recovery of parasites from naturally infected animals was made. Intracerebral inoculation was much less efficient than intraperitoneal. The use of the dye test in mice which had suffered asymptomatic infections was found to be an efficient method of detecting infection. The use of freezing in glycerine for the preservation of strains was made a routine procedure, and preservation for as long as one year was demonstrated. Dye tests run before, during and after malaria infection in man have shown no effect of this infection upon the serological findings.

Significance to the Program of the Institute:

The fact that the mode of transmission of toxoplasmosis remains unelucidated may be evidence of deficiencies in the fundamental knowledge of the life cycle of the parasite. Addition to the knowledge already available concerning

the occurrence of this parasite in man and animals may provide leads as to the mode of transmission.

Proposed Course of Project:

Further studies will be made of Toxoplasma infections in animals associated with man. The emphasis will be shifted to studies having possible bearing on transmission, such as studies of the occurrence of Toxoplasma in secretions and excretions. Infections with newly isolated strains rather than with possibly modified laboratory strains will be stressed, and investigations of the oral and nasal induction of infection will be made. Studies of the survival of parasites in tissues and under varying environmental conditions will be continued.

Project Subtitle:

The Chemotherapy of Toxoplasmosis.

Objectives:

The primary objective of this project is to evaluate the effect of known antitoxoplasmic agents in man and in animals and to find new drugs, particularly drugs less toxic to man than those now available and active against the chronic forms of the parasite. Secondarily, the objective is to study the fundamental physiologic mechanisms of the parasite as related to chemotherapy.

Methods Employed:

The effect of drugs of known activity such as pyrimethamine and sulfonamides is being studied in man in cooperation with local ophthalmologists and internists. Studies are also being made in laboratory animals and in domestic animals, including studies of the effect of metabolites in reversing drug action. A small scale screening program is maintained to investigate new agents upon a highly selective basis.

Major Findings:

Apparently successful results have been obtained in a number of instances in which pyrimethamine and sulfonamides have been used to treat presumed toxoplasmic uveitis or chorioretinitis. Further evaluation of the relative effect of sulfonamides has shown that sulfamethazine and sulfamerazine may be superior to sulfadiazine but that all are of the same order of activity. The action of Puromycin, previously reported to be moderately active against Toxoplasma infection, was reversed by the concomitant administration of the purine metabolite, adenine. Compared to the parent drug, the aminonucleoside of Puromycin was much more active. Pyrimethamine and Puromycin did not interact in their effect upon Toxoplasma even though they are believed to act upon related metabolic systems. Sulfonamides and pyrimethamine were found to be active against intracerebrally induced infections in chickens. A few drugs of minor activity were disclosed by the screening activity.

Significance to the Program of the Institute

Studies of the chemotherapy have yielded drugs which appear to be proving of practical value in the treatment of toxoplasmosis in man. The finding of synergism of pyrimidines and sulfonamides against Toxoplasma (along with previous knowledge of similar synergistic action in malaria and against bacteria in vitro) have led to investigations of possible synergistic effects against other organisms (ie. against coccidia of the domestic fowl and against human bacterial infections).

Proposed Course of Project:

Further evaluation of the effect of drugs in man will be carried out to the greatest extent possible. At the same time further studies in animals and possibly in tissue culture will be continued to study in greater detail the mode of action of the drugs. Attempts will be made to find other drugs which will act upon the same enzyme system as pyrimethamine and sulfonamides in order to impose further sequential blocks and thus improve antitoxoplasmic effect. Studies of purine analogs will be continued. Screening of compounds active against other infections will be continued upon a limited and highly selective basis.

Project Subtitle:

The Epidemiology of Toxoplasmosis.

Objectives:

To determine the mode of transmission of toxoplasmosis and the factors influencing the spread of this infection.

Methods Employed:

General epidemiological approaches are used to obtain information on various items such as age, sex, race, habitat, occupation, mental condition, housing and other pertinent factors in relation to the serological response of various population groups to the dye test for Toxoplasma antibodies. Parasitological and serological studies are done to determine to what extent wild or domestic animals may serve as a reservoir of human infections. Tests are being run to determine whether the infection spreads more rapidly in an institution than in the normal population.

Major Findings:

human

Studies on blood sera from Guatemala and Costa Rica have shown that toxoplasmosis is two to four times as frequent in Central America as it is in the United States, and that it occurs more often at altitudes below 5,000 feet than it does at higher elevations. Incomplete analysis of serological tests on 700 children in the Memphis area, six to nine years of age, indicates that antibodies occur in about six per cent. Incomplete serological studies on an urban Caucasian population suggest the possibility that young adult males have

Toxoplasma antibodies more frequently than do females of comparable ages. This is the first evidence seen of a sexual difference in a normal population group and the finding needs verification.

During intensive investigations around the slum-area premises where a fatal congenital case of toxoplasmosis presumably was contracted, Toxoplasma infection was found to be enzootic in the animal population of the immediate neighborhood. Parasites were frequently recovered from cats, mice, dogs, pigeons, ducks, and chickens, but not from wild birds. The infection was found in 37 per cent of the mice collected within the patient's home, but only rarely in mice from neighboring houses.

Comparable studies of animals from other areas revealed a high degree of Toxoplasma infection in cats from the Memphis animal pound and from a slum area similar to but several miles distant from the one where the human case of toxoplasmosis occurred. A lesser degree of infection was found in cats from Columbia, South Carolina. House mice from the Memphis slum area were apparently free of the infection.

Additional strong confirmative information has been obtained indicating that toxoplasmosis is closely correlated with certain cases of ocular disease.

A serologic study of the prevalence of Toxoplasma antibodies in the inmates of several institutions has been continued. Analysis of the data from one of these showed a significantly higher prevalence in male mental defective patients than in comparable female patients, and that this difference was due principally to very high prevalence in several male wards, possibly indicating a situation favoring the transmission of the disease.

Significance to the Program of the Institute

Toxoplasmosis is being more widely recognized as a disease entity and serologic testing shows that it probably is of widespread prevalence. The present epidemiological studies should serve to elucidate further such important points as how these parasites are transmitted, mode of entrance into the body, the importance of animals as reservoirs of the disease, and other factors which need to be known before an intelligent approach can be made to a control program.

Proposed Course of Project:

During the next year, serologic studies of population groups under investigation will be completed and supplementary samplings made to clarify points of question. Studies of the occurrence of Toxoplasma infection in possible host animals will be continued and from the serologic studies and the host studies clues will be sought as to the mode of transmission. Cooperative efforts with local physicians will be continued toward the end of finding cases of toxoplasmosis for epidemiological study and toward determining the relationship of toxoplasmosis to clinical findings. The relationship to mental disease and to institutional housing will be investigated further. Special studies will be made to test the hypothesis of oral transmission by the consumption of parasitized meat or other food.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-76
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$188,034	66,100	254,134	10.0	22.0	32.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	11	23	34	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

For the Columbia projects, the South Carolina State Hospital provides some facilities and active participation of certain staff members for much of the program concerned with the chemotherapy and epidemiology of parasites. The Pineland Home for Mental Defectives (Negroes) cooperates by making available sera for toxoplasmosis work and unusual cases of parasitic infections for study.

At the Memphis Laboratory, the University of Tennessee Medical School and the Veterans Administration Medical Training Group Hospital cooperate by the active participation of certain staff members in the toxoplasmosis work in the areas of case finding, clinical diagnosis, and treatment.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards & Publications 15. NIAID-76
Serial Number

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956.

Eyles, D. E. and Jones, F. E. The Chemotherapeutic Effect of Pyrimethamine and Sulfadiazine on Toxoplasmosis of the Norway Rat. Antibiotics and Chemotherapy. 5:731-734. 1956.

Jeffery, G. M. Relapses with Chesson Strain Plasmodium vivax Following Treatment with Chloroquine. Am. J. Trop. Med. and Hyg. 5:1-13. 1956.

Burgess, R. W. The Effect of Topically Applied DDT on Development of Plasmodium vivax and P. falciparum in Anopheles quadrimaculatus. Am. J. Trop. Med. and Hyg. 5:163-167. 1956.

Wettingfeld, R. F., Rowe, John, and Eyles, D. E. Treatment of Toxoplasmosis with Pyrimethamine (Daraprim) and Triple Sulfonamide. Ann. Internal Med. 44:557-564. 1956.

Young, M. D. and Freed, J. E. The Effect of Puromycin Against Entamoeba histolytica and Other Intestinal Parasites. Sou. Med. J. 49:537-538. 1956.

Eyles, D. E. and Coleman, N. Relationship of Size of Inoculum to Time to Death in Mice Infected with Toxoplasma gondii. J. Parasit. 42:272-276. 1956.

Eyles, D. E. Newer knowledge of the chemotherapy of toxoplasmosis. Ann. N. Y. Acad. Sci. 64:252-267. 1956.

Eyles, D. E. Toxoplasmosis: Summary and Challenge. Ann. N. Y. Acad. Sci. 64:275-277. 1956.

Jeffery, G. M. Blood Meal Volume in Anopheles quadrimaculatus, A. albimanus and Aedes aegypti. Exp. Parasit. 5:371-375. 1956.

Jeffery, G. M., Young, M. D. and Eyles, D. E. The Treatment of Plasmodium falciparum Infection with Chloroquine, with a Note on Infectivity to Mosquitoes of Primaquine- and Pyrimethamine-treated Cases. Am. J. Hyg. 64:1-11. 1956.

Rendtorff, R. C. Treatment of a Case of Taenia saginata Infection with Diphenhydramine. J. Parasit. 42:382. 1956.

Eyles, D. E., Coleman, N. and Cavanaugh, D. J. Preservation of Toxoplasma gondii by Freezing. J. Parasit. 42:408-413. 1956.

Gibson, C. L., Eyles, D. E., Coleman, N. and Smith, C. S. Serological response of a rural Negro population to the Sabin-Feldman cytoplasm-modifying test for toxoplasmosis. Am. J. Med. Hyg. 5:772-783. 1956.

Jeffery, G. M. Human Coccidiosis in South Carolina. J. Parasit. 42:491-495. 1956.

Jeffery, G. M. Intestinal Parasites in a Georgia Mental Hospital. J. Parasit. 42:553-555.

Gibson, C. L. Distribution of Toxoplasma Antibodies in Comparable Urban and Rural Groups. Pub. Hlth. Rep. 71:1119-1123. 1956.

In Press

Young, M. D. The Response of Plasmodium malariae Infections to Pyrimethamine (Daraprim). Am. J. Trop. Med. and Hyg.

Wolcott, G. B. Chromosome Studies in the Genus Plasmodium. J. Protozool.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Dr. M. D. Young accepted a reappointment of five years to the WHO Expert Advisory Panel on Malaria.

Drs. G. M. Jeffery and R. W. Burgess received the Jefferson Award of the South Carolina Academy of Science for an outstanding paper entitled "Infection of Mosquito Larvae with the Virus of Lymphocytic Choriomeningitis" which was presented at the annual meeting.

Dr. G. B. Wolcott was elected to the Council of the South Carolina Academy of Science.

Dr. D. E. Eyles was invited to present a paper on the treatment of toxoplasmosis to the Eighth International Congress of Paediatrics in Copenhagen, July 22-27, 1956.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-77
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Protozoal Diseases
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the Biology and Pathogenesis of Protozoan Parasites of Man
PROJECT TITLE

7. Dr. Leon Jacobs
PRINCIPAL INVESTIGATOR

8. Dr. H.D. Baernstein, Dr.T.A.Burch, Miss L.V. Reardon, Miss M.L. Melton, Miss M.K. Cook, Mr. M.L. Lunde
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

Project Subtitle:

Studies on amoebiasis

Objectives:

The long-range objectives of investigation in amoebiasis are: Information on the growth factors of Entamoeba histolytica in pure culture as prerequisite for metabolic investigations of the parasite, clarification of the pathogenesis of the parasite as a basis for investigating the infectivity of strains and of effective chemotherapy and control.

Methods Employed:

The methods include the development, maintenance, and use of cultures of amoebae with single selected species of bacteria as well

as cultures with a number of species of bacteria, the latter for the production of cysts in vitro; cultures without bacteria but with Trypanosoma cruzi; cultures with minced embryonic tissues and with cell-free homogenates of tissues; employment of specially devised techniques in biochemistry for studies on growth factors; the use of techniques of microisolation and microculture.

Cooperation is extended to physicians of the Clinical Center, NIH, in investigations of patients with amoebiasis by clinical methods as a basis for diagnosis, prophylaxis, and therapy.

Major Findings:

Amoebae which have been cultivated with Trypanosoma cruzi differed in their nutritional requirements from the same strain (F-22) which had been adapted to chick embryo medium. There was some evidence that prolonged cultivation in T. cruzi medium produced a gradual change in the requirements of E. histolytica. When minced chick embryo medium was stored at 24° or 38° C., it quickly lost its ability to support growth of the amoeba; when frozen at -10° C. the medium retained activity for 24 days. This indicates that autolysis of complex factors is an important factor in relation to amoebic growth. Some of the lost activity of the medium could be replaced by the addition of glucose oxidase (in the form of Notatin, a flavoprotein derived from Penicillium mold), indicating that the oxygen uptake of the subcellular particles in the medium had been inactivated on storage. Notatin in the presence of heated particles supported, and in the presence of unheated particles, stimulated growth of amoebae. Notatin must also contribute heat-stable factor(s) because, even after boiling, it produced a stimulation of amoebic growth, in the presence of unheated particles, similar to that produced by the unheated enzyme. The stimulatory effect of Notatin may be due to its ability to remove oxygen from the system (in the case of the unheated enzyme) or possibly also to its containing heat stable factors such as flavin-adenine-dinucleotide which is its prosthetic group. The subcellular particles in the medium are also capable of removal of most, if not all, of the oxygen in the medium; hence growth of amoebae could proceed in the presence of unheated medium and heated Notatin. Further evidence that growth of E. histolytica may depend on an oxygen uptake system such as succinoxidase was obtained by addition of a rather specific inhibitor, malonate, which completely inhibited growth. The inhibition could be of the oxygen uptake mechanism of the subcellular particles or of the amoeba itself. Dialyzed serum loses some of its growth-promoting properties; these can be restored by the addition of a mixture of water-soluble vitamins. Rabbit serum added to the horse serum already in the medium stimulated growth of amoebae, but b itself did not replace horse serum. Hemoglobin did not enhance growth.

Significance to the Program of the Institute:

Analysis and definition of the growth factors required by Entamoeb histolytica is expected eventually to yield considerable information not only on this parasite but in relation to other organisms. In regard to the amoeba, the growth without other organisms will allow investigations into the physiology of the parasite and of its various strains, thus

possibly providing criteria for differentiating strains other than by the present inadequate bases of morphology and virulence in unnatural experimental hosts. In regard to general microbiology, the solution of the problem of amoebic growth may be expected to furnish information relative to the requirements of many other fastidious organisms, bacteria as well as protozoa.

Proposed Course of Project:

The studies on growth of E. histolytica will be continued toward the goal of strictly axenic cultivation, particularly in macrocultures. Analysis of the oxygen requirements of the amoeba will be continued, and the complex medium thus far employed will be dissected so that definition of specific growth factors can be achieved. Studies on changes induced by selected microbial associates will also be continued, as well as on the effects of encystation and excystation on the adaptibility of the amoeba to culture and on its pathogenicity. It is expected also that some collaborative work will be initiated with the NINDB in regard to the problem of a newly described ocular inflammation which has been correlated with amoebic infection as revealed by the presence of parasites in the intestine or by positive complement fixation tests and by response to anti-amoebic therapy. Continued collaboration with LCI, NIAID, is also on the agenda, to obtain as much information as possible from clinical studies on the organism in man.

Project Subtitle:

Genital Trichomoniasis

Objectives:

To investigate: (1) Disturbances attributable to Trichmonas vaginalis in the urogenital tract of females; (2) the pathogenesis of T. vaginalis alone or associated with other organisms; (3) transmission; (4) the relation of T. tenax and T. hominis to T. vaginalis; (5) incidences and epidemiology; (6) the possible relation to the diagnosis of malignancies by vaginal cytology; (7) chemotherapy.

Methods Employed:

Disturbances in the urogenital tract are investigated by clinical observation and study of associated bacteria and fungi and changes in vaginal physiology as indicated by pH, percentage of cornified cells, changes in glycogen content etc., before and after elimination of Trichomonas vaginalis by means of chemotherapy. Some in vitro drug testing has been undertaken. Studies on transmission include comparative incidences in husband and wife, other household contacts, experiments with contaminated fomites, and examination of specimens from the mouth and the intestinal tract. Other epidemiological investigations are conducted by means of questionnaires, interviews, and examination of specimens as described above. Data are obtainable from a large number of women employees of NIH and other places, and from physicians in private practice.

Major Findings:

Data from a survey of healthy white women at NIH showed a positive correlation between vaginal discharge and/or genital itching and the occurrence of T. vaginalis. Thirty-nine percent of those who admitted such a discharge and/or itching had T. vaginalis, while only 4 percent of those denying the condition had the infection. It was shown that there was essentially no difference of candidiasis in the two groups, but that in women admitting a discharge prior to but not including the past 12 months approximately twice the prevalence of the fungus was found as in the other 2 groups. These data indicate that Candida originally does not cause symptoms but when symptoms are produced, they are not of such long duration as with trichomoniasis.

Ten of 41 patients with Trichomonas vaginalis have been rendered parasite-free for a period of from 3 to 9 months after treatment with one or more of 9 commercially available trichomonicidal preparations. The most effective was a combination of vaginal and oral Aureomycin which "cured" 5 of 14 patients treated. Nine additional patients were treated with Proprion Gel concomitantly with Aureomycin to combat an associated Candida infection. Only one of the 9 patients became parasite-free and she did not receive any Proprion Gel until she was nearly through with the course of Aureomycin. The possible antagonistic effect of Proprion Gel and Aureomycin was tested in vitro against Trichomonas vaginalis and it was observed that Proprion Gel had essentially no trichomonicidal effect when used alone but when added to Aureomycin the trichomonicidal effect of the latter appeared to be greatly increased. The reason for this discrepancy is not apparent. It points up the differences which can occur in results of in vitro and clinical trials.

Coincident with the presence of vaginal trichomoniasis, an increase in the pH of the vaginal secretions has been observed in 25 women, 13 colored and 12 white. The greatest shift has been observed in the latter. There is also a correlation between a high pH and an alteration in the bacterial flora away from the predominance of lactobacelli. Also, quantitative studies on the condition of the vaginal epithelium, using as a criterion the cornification, have shown that the normal picture is seen rarely or not at all in infected women. For instance, in the middle phase of the estrus cycle when high cornification is expected, low figures have been found; the reverse has occurred in early and late phases.

Using cultural methods, a much higher prevalence of trichomoniasis has been found in sex partners of infected women than was revealed by direct examination.

Significance to the Program of the Institute:

Trichomonas vaginalis is one of the most prevalent parasites of man in America today and is responsible for symptoms of varying severity in nearly every infected patient. Investigations on this parasite and its associated conditions may enable the Institute to contribute to the lowering of the number of infected patients and decreasing the amount of physical and nervous discomfort caused by it.

Proposed Course of Project:

More detailed information will be sought on the following points: (1) The clinical appraisal of therapy in alleviating symptoms in infected women; (2) the alterations in bacterial flora associated with the trichomonad in various degrees of symptomatology; (3) the comparison of vaginal cytology in series of infected women before and after therapy, and in uninfected women; (4) prevalence of infections in consorts of infected women, and the persistence and chemotherapeutic control of the infection in the male. In addition, improvements in cultural techniques and laboratory criteria for evaluating strain differences will be sought. Serological procedures will be exploited for studies on the relations of the trichomonad parasites of man, and for their possible usefulness in diagnosis.

Project Subtitle:

Studies on Toxoplasmosis

Objectives:

The elucidation of the biology of Toxoplasma and related organisms, especially transmission and reservoirs of the infection for man; the characterization of strain differences, of factors involved in susceptibility and immunity; the development of an adequate and practicable diagnostic test for systemic toxoplasmosis, the development of special criteria for the definitive diagnosis of ocular toxoplasmosis; the description of the lymphadenopathic and ocular forms of human infection, and the therapy and management of the ocular form as indicated by experimental studies; the description of certain factors in the physiology of the parasite in vitro especially as they relate to drug action and immunity.

Methods Employed:

The techinques are virological methods adapted for this fragile intracellular parasite. They include the range of animal inoculation procedures and in addition injections into the anterior and posterior chambers of the eye. Tissue culture techniques are employed, and the unique in vitro dye test is used for titration of serum antibodies.

Collaborative clinical and laboratory studies are in progress with the Wilmer Institute of Johns Hopkins University and the Clinical Center of NIH. A similar project of lesser magnitude is conducted with Eye Clinic of Walter Reed Army Hospital.

Major Findings:

A new serological procedure for the diagnosis of ocular toxoplasmosis has been developed. This is a hemagglutination test, using a protein antigen from lysed toxoplasmas. The test has been found to give titers comparable to those in the in vitro dye test, and to become positive more rapidly than the c. f. test. It has been shown to be specific and reliable; over 20 different preparations of antigen have given similar results.

Tissue culture tests of drug action have shown that pyrimethamine is stored or bound in the host cells in vitro so that even after drugged medium is replaced with normal medium, proliferation of inoculated parasites is inhibited. The inhibition time is related to the original concentration of drug and to the time after drug removal. Also, by the use of alternate passages in mice and tissue cultures, a strain of Toxoplasma 300 times more resistant to pyrimethamine in vitro has been developed. This resistance, however, has not been equally demonstrable in in vivo tests in mice. The anti-Toxoplasma action of pyrimethamine in vitro appears to occur inside the cells; the drug has not been found to prevent invasion of cells by free parasites.

Further studies of Toxoplasma antibodies in cases of granulomatous uveitis, studied in a collaborative project with the Wilmer Institute, indicate that at least 35 percent of such cases can be attributed to toxoplasmosis. Some cases responded well to anti-Toxoplasma therapy.

A spontaneous case of acquired toxoplasmosis was studied over a long period of time. Parasites were isolated twice from lymph nodes and once from muscle. The salient features about this case were: (1) The development of an ocular lesion which appeared about 3 months after the onset of systemic disease and which responded rapidly to therapy with pyrimethamine and sulfadiazine. This is evidence that ocular lesions can occur in acquired as well as in congenital infections, and is the best documented case of this sort. (2) Failure to obtain radical cure, i.e. complete removal of parasites, when therapy is started late in infection. This is believed to be due to the presence of pseudocysts, or latent forms of the parasite, which are not destroyed by the drugs.

Experimental ocular toxoplasmosis of rabbits has been controlled with doses of 100 mgm./kg. of DDS, diamino diphenyl sulfone, as well as with a like dose of pyrimethamine. Of 3 symmetrical triazines tested, one showed some ability to control experimental ocular toxoplasmosis.

The pseudocyst form of Toxoplasma is capable of surviving longer in dead tissue than is the proliferative form. In studies to evaluate the possibility that man acquires the infection by ingesting infected flesh, dye tests done on 46 serums, mostly from people over 40 years of age who had never eaten flesh or had not eaten any for over 20 years, revealed positives in 21.6 percent. High titers were obtained in people who had not eaten flesh for 30 or more years. These results point to other means of acquiring Toxoplasma infection than by meat.

Despite high antibody titers, guinea pigs immunized by dead antigens are not entirely resistant even to relatively small challenge inocula. Studies on parabiotic rats united after immunization of one with a live inoculum, and challenged after antibody titers are similar in both members of a pair, point to the possibility that local tissue immunity plays a role in the spread or confinement of a challenge inoculum.

Sarcocystis muris has been established in laboratory mice, for studies on its mode of transmission and on its relation to the specificity of the dye test. The infection was transmitted to mice which were fed the feces of other mice given Sarcocystis-infected tissue per os.

The infection did not develop in the latter mice.

Significance to the Program of the Institute:

There is ample evidence of the importance of Toxoplasma in ocular disease. What is still needed is a definitive method of diagnosis for toxoplasmic uveitis. Accomplishments along this line can be expected to be of use in diagnosis of other chronic ocular infections.

The development of the hemagglutination test may result in considerable stimulation of work on toxoplasmosis. It is more practicable than the dye test, which because of its special requirements and difficulties, has been performed in only a few laboratories.

Toxoplasma offers continued opportunity for basic study of the cellular and humoral factors in immunity, both in vivo and in vitro, in the susceptibility of hosts, the resistance and pathogenesis of strains, and host-parasite relations at the level of cellular metabolism. The identification of antigens responsible for dye test antibodies as distinct from complement fixing factors is also of broad significance.

Proposed Course of Project:

Attention will be devoted to the following points during the next calendar year: (1) The localization of dye-tagged immune globulins in experimental uveitis, with a view to using such a method for definitive diagnosis of toxoplasmic uveitis in humans; (2) serial dye test titers on normal individuals to obtain a base line for evaluating fluctuations in antibody titers seen in cases of presumed ocular toxoplasmosis; (3) exploitation of the hemagglutination test in various forms of toxoplasmosis to obtain information on the time of appearance and persistence of hemagglutinating antibodies relative to dye test and c.f. antibodies; (4) development of pure antigens for this test and for possible use in therapy of ocular infections; (5) the transmission of Toxoplasma, especially the importance of meat as a source of human infection; (6) tissue culture studies of Toxoplasma for morphology and method of reproduction, as well as for information on the mechanism of action of pyrimethamine and other drugs, and on drug storage and development of resistance in cells in vitro; (7) comparison of the effects of immunization with live and dead agents; (8) study of Sarcocystis infections in mice and other animals to define more closely its method of transmission and exploit, if possible, such knowledge in relation to the transmission and serology of toxoplasmosis.

Form No. ORP-1
October 1956
(Attachment 1)

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-77
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$92,461	$42,500	$134,961	8.0	5.0	13.0

	BUDGETED POSITIONS			PATIENT DAYS
	PPROF	OTHER	TOTAL	
FY'57	9	6	15	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

For the project on Studies on Toxoplasmosis, Ophthalmology Branch, NINDB paid the salary of Miss M. K. Cook through June 1956.

Wilmer Ophthalmological Institute, Johns Hopkins Hospital, Baltimore, Md. provided clinical specimens for the same project.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards & Publications

15. NIAID-77
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Jacobs, L.; Naquin, H.; Hoover, R.; and Woods, A.C.: A comparison of the toxoplasmin skin test, the Sabin-Feldman dye tests, and the complement fixation tests for toxoplasmosis in various forms of uveitis. Bull. Johns Hopkins Hosp. 99, 1-15, July 1956.

In Press

Baerrstein, H.D.; Rees, C.W.; and Bartgis, I.L.: The rate of reproduction of Entamoeba histolytica in microcultures from inocula of single trophozoites in cell-free medium prepared from embryos of the chick. J. Parasitol.

Jacobs, L.: Ocular toxoplasmosis: Laboratory contributions to diagnosis and chemotherapy. Proc. VIII Internat. Cong. Tropical Med.

Jacobs, L. and Lunde, M.L.: A hemagglutination test for toxoplasmosis. J. Parasitol.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Leon Jacobs received the Barnett Cohen Award of the Maryland Branch, American Society of Bacteriologists, April 14, 1956.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-76
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Chemotherapy
SECTION OR SERVICE

5. _____
LOCATION (IF OTHER THAN BETHESDA)

6. Chemotherapy of Parasitic Diseases
PROJECT TITLE

7. Dr. G. Robert Coatney
PRINCIPAL INVESTIGATOR

8. Dr. J. Greenberg, Dr. J. Sherman, Dr. G. Luttermoser, and Miss D. J. Taylor
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

Project Subtitle:

Drug Resistance in Plasmodia

Objectives:

To study the methods by which resistance to therapeutic agents is acquired by animal parasites; the stability of resistance once acquired; its heritability; the interaction between susceptible and resistant parasites through the sexual cycle; the relation between resistance and other measurable biological properties of the parasite such as sexuality and tissue preference. To study the influence of heritable characteristics of the host on the biological properties of the parasite.

Methods Employed:

Using Plasmodium gallinaceum, strains are developed with unique combinations of properties involving drug-resistance, virulence, and sexuality. These strains are hybridized systematically and recombina-

tions recovered and analyzed. P. berghei is used in inbred strains of mice and their hybrids. Tissue preferences of the parasite in these animals are observed and compared.

Major Findings:

Previous studies of Plasmodium gallinaceum made resistant to Daraprim had shown that this phenomenon persisted through mosquito passages. Continuing studies have shown that: (1) genuine hybrids can be produced, (2) these hybrids are resistant also, and (3) this resistance can be transferred through the mosquito.

Significance to the Program of the Institute:

From a practical point of view, information on drug resistance is of value to those interested in programs of mass therapy, malaria and tuberculosis for example. From the viewpoint of basic research the plasmodia are invaluable tools in studies involving the genetics of microorganisms. Very few other pathogenic organisms have a sexual cycle, so the genetic transfer of drug resistance can only be inferred and cannot be studied in terms of number of genes involved and the location and interrelation of such genes. Many characteristics, such as pathogenicity and tissue preferences, are intimately related to drug activity. For example, the therapeutic failure of such compounds as quinine is now known to be related to its inability to kill parasites in certain stages of their development. There is reason to suppose that plasmodia are not unique in this respect. Furthermore, it has been shown that the virulence of plasmodia can be modified by certain experimental procedures. The practical and theoretical implications of these observations are readily apparent.

Proposed Course of Project:

This project was discontinued at the end of June in favor of basic studies on the mode of action of therapeutic agents with particular emphasis on Puromycin, in line with the redirection of the program of the entire Section.

Project Subtitle:

Experimental Chemotherapy

Objectives:

To discover new and more effective therapeutic agents for the treatment of amoebiasis and schistosomiasis.

Methods Employed:

(a) Weanling guinea pigs are inoculated intracecally with a virulent strain of Entamoeba histolytica from cultures and then treated with the test compounds. Most compounds must be evaluated both for toxicity and their ability to prevent or cure the infection.

(b) Mice are infected with known numbers of Schistosoma mansoni cercariae. Treatment is begun, using synthetic compounds and natural products, about the time worms are sexually mature. Evaluation of test compounds is based on the number of eggs in the liver, and the abundance of dead and live worms as compared to the same factors in untreated mice and those treated with a standard drug.

Major Findings:

(a) Only six compounds related to Puromycin were available for testing; none was as active as Puromycin against E. histolytica. Preliminary experiments showed that a group of benzimidazole compounds were worthy of critical comparison with Puromycin. Amoebae together with bacteria, grown in a simplified liquid medium proved useful as an in vitro method for evaluating small quantities of compounds for amoebacidal activity and may also be useful in determining activity on the bacteria. Serial passage of amoebae through guinea pigs has proved to be the most successful method for enhancing the virulence of the parasite.

(b) Among the compounds tested, those containing antimony were the most active against S. mansoni. When various adjuvants, such as hydroxycarboxylic acids or their salts, were combined with the most active of these compounds, activity was not enhanced. However, when the same adjuvants were combined with antimony trioxide, inactive by itself, curative activity was demonstrated. This curative activity was found to depend on the pH and the presence of free hydroxyl or sulphydryl linkage.

Significance to the Program of the Institute:

(a) There is a continuing need for a more effective and nontoxic amoebacide. This can be discovered both through mass screening of miscellaneous compounds and through the logical exploitation of chemical structures known to have some activity against the parasite. Puromycin appears to be a logical compound for exploitation because of its tripartite chemical structure and its peculiar antibiotic spectrum. In addition, further studies on the virulence or pathogenicity of amoebae are needed both from the practical standpoint of maintaining an adequate test system for therapeutic studies and from a basic viewpoint of answering fundamental questions concerning the carrier state versus active amoebiasis.

(b) Treatment of schistosomiasis remains hazardous and difficult; the discovery of more effective and safer therapeutic agents is a practical necessity. The rationale of the present program is twofold: (1) mass screening of chemical compounds and (2) systematic exploitation of modifications of chemicals such as Miracil-D and antimonials.

Proposed Course of Project:

Further evaluation of compounds for the treatment of amoebiasis and schistosomiasis will be carried out but with the main emphasis along lines of drug action.

Project Subtitle:

Chemical Pharmacology and Toxicology

Objectives:

To determine basic pharmacological and toxicological properties of therapeutic compounds as well as methods for the assay of such compounds and their degradation products in biological systems, their distribution, degradation and excretion, and preferred methods of administration.

Methods Employed:

Standard pharmacological methods are used to determine the toxicity and pharmacodynamics of compounds in various hosts and their effects on various organs and systems. Chemical and physical methods are devised for the extraction and identification of therapeutic agents and their degradation products from biological systems. The methods used include differential extraction and precipitation, chromatography, visible and ultraviolet spectrophotometry.

Major Findings:

Continued efforts toward development of a satisfactory assay for the antibiotic, Puromycin, in biological fluids followed two directions. The first involved paper chromatography of the agent followed by elution and quantitative determination by ultraviolet spectrophotometry. This has thus far proved unsuccessful due to high variability in recovery of known quantities of the drug from the filter papers tested. The second line of endeavor involved the use of several microorganisms in different media. This, too, was unsuccessful as the drug was not sufficiently active at desirable concentrations to provide reproducible results.

Solubility determinations were made on 13 acid salts of the antimalarial, Daraprim, previously prepared in this laboratory. The most soluble was the sulfamate (5.0461 gm./100 ml. H_2O at 25° C) while the least soluble was the citrate (0.1173 gm./100 ml.H_2O). This compares with a Daraprim solubility of 0.0035 gm./100 ml. H_2O.

Efforts in this laboratory and several commercial laboratories resulted in the formulation of a stable mixture of table salt and the antimalarial, chloroquine, for possible use as a prophylactic measure in malaria-ridden areas of the world. Simple spectrophotometric assay methods developed in this laboratory permitted the rapid and accurate quantitation of either chloroquine or pyrimethamine in such drug-table salt mixtures.

Significance to the Program of the Institute:

Information contributed by studies in chemical pharmacology are of prime importance towards development of rational approaches to problems in chemotherapy. Such information is derived from examination of drug metabolism and activity in parasite as well as in host and on the interrelations

resulting from infection. The elucidation of modes of drug action by such means is essential in the replacement of present empirical methods. Equally important to the field are the data obtained from animal experimentation on the toxicological and pharmacological properties of potential therapeutic compounds. Evaluation of such information is a prerequisite for the design of safe and effective clinical trials.

Proposed Course of Project:

This project was allowed to lapse after June 30th due to loss of professional personnel (Dr. Bond and Dr. Sherman) through transfer to other Institutes. Plans now under way call for reactivation of the project after January 1, 1957, with emphasis on the toxicology, method of assay, and pharmacodynamics of Puromycin and related compounds.

Project Subtitle:

Clinical evaluation

Objectives:

To determine whether Chesson strain vivax malaria could be suppressed completely by administering antimalarial drugs, pyrimethamine and chloroquine, in combination with common table salt taken ad lib.

Methods Employed:

Inmate volunteers were fed at a special mess where the food is seasoned in the kitchen, and at the table if desired, with a salt-drug mixture. After approximately two weeks, each "eating" volunteer and each control was infected with malaria by the bites of 10 heavily infected mosquitoes. The procedure was repeated on two subsequent occasions one week apart following which participants continued on the special mess until 5 days after all controls had shown malaria.

Major Findings:

Pyrimethamine at a calculated dosage of 25 mg. per week, and chloroquine at a calculated dosage of 300 mg. (base) per week each produced complete suppression of malaria without evidence of toxicity, when used as a salt-drug mixture.

Significance to the Program of the Institute:

The demonstration that antimalarial drugs can be given successfully in a universal commodity such as common table salt makes control of this disease economically feasible in areas where residual insecticide sprays are not successful.

Proposed Course of Study:

Objective has been realized and project will be closed out.

Form No.ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-78
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$76,721	26,900	103,621	4.0	6.9	10.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	5	6	11	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

For the Clinical Evaluation project the Laboratory of Nutrition and Endocrinology, NIAMD, cooperated by performing chemical tests; WHO and Army Research and Development Board provided funds; Bureau of Prisons, Department of Justice provided facilities and prisoner volunteers.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-78
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Sherman, J. F.: Enhancement of the central nervous system effects of strychnine and pentobarbital by diphenhydramine. Science, 123, 1170-1171, June 1956.

In Press

Greenberg, J. and Kendrick, L. P.: Parasitemia and survival in inbred strains of mice infected with Plasmodium berghei. J. Parasitol.

Greenberg, J. and Kendrick, L. P.: Some characteristics of Plasmodium berghei passed within inbred strains of mice. J. Parasitol.

Coatney, G. R.: Introduction of Leon Herbert Schmidt as the Charles Franklin Craig Lecturer. Am. J. Trop. Med. & Hyg.

Luttermoser, G. W. and Bond, H. W. : Anthelmintic activity of the fruits of Diospyros mollis (Maklua) and tests for activity of other persimmons. Proc. Helm. Soc.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Dr. Joseph Greenberg was awarded a Senior Postdoctoral Fellowship by the National Science Foundation.

Dr. George Luttermoser was appointed a member of the United States Pharmacopeia XVI Panel on Parasitology.

Dr. G. Robert Coatney received the Alumni Achievement Award at the 1956 Commencement exercises of Iowa State College.

Miss D. Jane Taylor was elected to active membership in Sigma Xi.

Dr. G. Robert Coatney accepted a reappointment of five years to the WHO Expert Advisory Panel on Malaria.

Dr. G. Robert Coatney was elected Vice President of the American Society of Tropical Medicine and Hygiene.

Dr. G. Robert Coatney was reappointed Visiting Lecturer in Tropical Public Health, Harvard School of Public Health.

Dr. G. Robert Coatney was reappointed Visiting Professor in the Department of Preventive Medicine and Public Health, Howard University, School of Medicine.

Dr. G. Robert Coatney was reappointed Visiting Lecturer on Malaria, Georgetown University, School of Medicine.

Dr. G. Robert Coatney was reappointed Chairman of the Editorial Board of the American Journal of Tropical Medicine and Hygiene.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-79
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH, OR DEPARTMENT

4. Section on Physiology
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA)

6. Fundamental Physiological and Biochemical Studies on Parasites, Intermediate Hosts, and Parasitized Animals
PROJECT TITLE

7. Dr. Theodor von Brand
PRINCIPAL INVESTIGATOR

8. Dr. E. C. Weinbach, Dr. W.L. Newton, Dr. T.I. Mercado, Mrs. E.J. Tobie
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

Project Subtitle:

Physiology and Biochemistry of Parasites

Objectives:

The objective of this part of the program is to provide knowledge on the chemical composition and metabolism of parasites.

Methods Employed:

The respiration of parasites is studied in various media and under the influence of a variety of inhibitors. The chemical composition of parasites is investigated by application of a variety of techniques, such as extraction with different solvents, fractionation of extracts, paper chromatography, and paper electrophoresis. Culture methods are developed for trypanosomes that do not readily develop *in vitro*.

Major Findings:

Characterization of respiration of Echinococcus granulosus scolices. Demonstration of a polysaccharide consisting of galactose and glucosamine in Echinococcus granulosus. Demonstration of a sterol in Trypanosoma cruzi. Finding of a new filarial worm in dogs. Establishment of a culture of Trypanosoma congolense.

Significance to the Program of the Institute:

The studies on metabolism of parasites are essential for an understanding of the pathogenesis of parasitic diseases in which two living organisms interact. The studies on chemical composition are significant as a basis for an understanding of the antigenic properties of parasites. All these studies have a direct bearing on the development of a rational approach to chemotherapy.

Proposed Course of Project:

The investigation of the chemical constitution of parasites, especially trypanosomes, will be continued with special emphasis on lipids and amino acids. The pyruvate metabolism of trypanosomes will be investigated. The cultivation of trypanosomes that are difficult to grow in vitro will be continued.

Project Subtitle:

Physiology and Biochemistry of Intermediate Hosts

Objectives:

The objective of this part of the program is to study the basic metabolic relations of snails transmitting human blood flukes, the mechanism of action of molluscacides, and the possibility of snails developing resistance to molluscacides.

Methods Employed:

Desiccation and starvation studies are conducted on snails in collaboration with the Section on Helminthic Diseases. Heart rate, respiration, quantitative behavior of polysaccharides, lipids, lactic acid, and volatile fatty acids are followed during periods of desiccation and starvation in water. In cooperation with the Section on Aging, Laboratory of Psychology, NIMH, phosphate metabolism is studied in the brain and liver of senescent rats. Successive generations of snails derived from selected survivors of gradually increased concentrations of sodium pentachlorophenate are tested for changes in susceptibility.

Major Findings:

Desiccating snails survive by reducing their metabolism, but the latter remains aerobic. Snails starving in water reduce their metabolic rate to a lesser degree than desiccating ones. The important exergonic process of oxidative phosphorylation declines markedly in isolated mito-

chondrial preparations of certain senescent molluscan and mammalian tissues. Snails have developed a slight but significant amount of resistance to sodium pentachlorophenate.

Significance to the Program of the Institute:

The studies are in part contributions to comparative physiology and biochemistry, but also have direct implications on practical problems. The difficult situation encountered in some areas in combating snails undergoing desiccation requires knowledge of the desiccation physiology. The biochemical analysis of tissues from aged organisms is a contribution to problems in gerontology. The work on resistance of snails to molluscacides is essential to anticipate difficulties similar to those encountered with insecticides.

Proposed Course of Project:

The physiological studies on desiccating and starving snails will be discontinued early in 1957 and the personnel will be shifted to the other problems described under the two other subtitles. The work on aging tissues will be continued by making a more detailed study of the decline in oxidative phosphorylation with particular attention to the site of the biochemical lesion (whether substrate dehydrogenases or respiratory chain defect). The resistance studies will probably be terminated during 1957.

Project Subtitle:

Pathological Physiology

Objectives:

The objective of this part of the program is to elucidate the pathogenic action of parasites on their hosts and the behavior of parasites in vivo.

Methods Employed:

Infected (Plasmodium berghei) and noninfected rats (both starving and fructose-fed) are treated with steroids. Glycogen and lipids are assessed quantitatively and histochemically and the suprarenals are studied histologically. The blood pyruvate level of rats is studied quantitatively. Lesions of the central nervous system are induced in trypanosome-infected rats by producing relapses with the help of subcurative doses of the amino nucleoside of Puromycin. Isolated mitochondria are prepared from mammalian tissues by mechanical disruption of the cells in suitable media and separation of the subcellular particulates by differential centrifugation. Aerobic and anaerobic exergonic reactions are studied with such preparations obtained from animals in various pathological states (aging, treatment with uncoupling compounds, etc.).

Major Findings:

A partial reversal of the pathological glycogen distribution in livers of Plasmodium berghei-infected rats has been observed under the influence of steroids. A marked increase in lipid content of livers has been observed in

infected rats and the distribution of this fat is the opposite of the pathological glycogen distribution. The blood pyruvate level of trypanosome-infected rats stays about normal, even though the parasites produce very large amounts of that acid, while in rats infected with malarial parasites an increase in blood pyruvate occurs. Contrary to many published reports, it was found that isolated rat liver mitochondria are capable of oxidative phosphorylation after storage at low temperatures for periods up to 72 hours. Other evidence of tightly coupled mitochondria was the observation that fresh preparations required no hexokinase-glucose trapping system, nor fluoride, to demonstrate maximal P:O ratios.

Significance to the Program of the Institute:

The studies tend to clarify the pathogenesis of parasitic diseases which still is quite obscure. Obviously, such a knowledge is of considerable importance for an understanding of the disease process and for therapeutic measures.

Proposed Course of Project:

The study of malaria-infected rats is practically completed and only very few additional data are required. As soon as these are obtained the work on malaria will be terminated. It is proposed to study by the above methods the pathology of liver function in other parasitic infections and to extend the investigation to the level of oxidative phosphorylation in order to study whether the biochemical lesions found in malaria are specific for that disease or whether they also occur in other infections, thus indicating a nonspecific cause. A more detailed study of the stability of mitochondrial aerobic phosphorylation with respect to substrate variation, phosphate acceptors, and morphological changes of aged mitochondrial preparations will be undertaken.

Form No. ORP-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-79
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$73,807	25,850	99,657	5.0	7.0	12.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	5	8	13	

13. BUDGET ACTIVITY:

RESEARCH	/X/	ADMINISTRATION	/__/
REVIEW & APPROVAL	/__/	PROFESSIONAL & TECHNICAL ASSIST-ANCE	/__/
BIOLOGIC STANDARDS	/__/		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Section on Aging, NIMH, provides all senescent rats used in Physiology and Biochemistry of Intermediate Hosts project and collates their data with LTD's.

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE --- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards & Publications 15. NIAID-79
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

von Brand, T. and Agosin M.: The utilization of Krebs cycle intermediates by the culture forms of Trypanosoma cruzi and Leishmania donovani. J. Infect. Dis., 95,274 - 279, November - December 1955.

von Brand, T. and Mercado, T.I.: Quantitative and histochemical studies on glycogenesis in the liver of rats infected with Plasmodium berghei. Exper. Parasitol; 5, 34-47, January 1956.

Mercado, T.I.; Trembley, H.L.; and von Brand, T.: Observations on the oxygen consumption of some adult mosquitoes. Physiol. Compar. Oecol., 4, 200 - 208, July 1956.

Newton, W.L. and Wright, W.H.: The occurrence of a dog filariid other than Dirofilaria immitis in the United States. J. Parasitol; 42, 246 - 258, June 1956.

Tobie, E.J. and Highman, B.: Influence of the aminonucleoside of Puromycin on the course and pathology of trypanosome infections in rabbits and mice. Am.J.Trop. Med. Hyg., 5, 504 - 515, May 1956.

Weinbach, E.C. and Nolan, M.O.: The effect of pentachlorophenol on the metabolism of the snail Australorbis glabratus. Exper. Parasitol., 5, 276 - 284, May 1956.

Agosin, M. and Weinbach, E.C.: Partial purification and characterization of the isocitric dehydrogenase from Trypanosoma cruzi. Bioch. Biophys. Acta, 21, 117 - 126, July 1956.

Weinbach, E.C.: Pentachlorophenol and mitochondrial adenosinetriphophatase J. Biol. Chem,, 221, 609 - 618, August 1956.

Weinbach, E.C.: The influence of pentachlorophenol on oxidative and glycolytic phosphorylation in snail tissue. Arch. Bioch. Biophys., 64 , 129 - 143, September 1956.

von Brand, T.: Beziehungen zwischen Stoffwechsel und taxonomischer Einteilung der Säugetiertrypanosomen. Zool. Anz., 157, 119 - 123,1956.

In Press

Agosin, M.; von Brand, T.: Rivers, G.F.; and McMahon, P.: Studies on the metabolism of Echinococcus granulosus.1. General chemical composition and respiratory reactions. Exper. Parasitol.

von Brand T.: Collaboration between basic and medical sciences. Anales Univer., Chile.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATIONS TO THIS PROJECT DURING CALENDAR YEAR 1956:

Dr. T. von Brand assumed the chairmanship of the Review Committee of Experimental Parasitology.

Dr. W.L. Newton was appointed to the NIAID Fellowship Specialty Board.

Dr. W. L. Newton was selected as one of the two scientific members of the Advisory Board of the NIH Revolving Fund.

Dr. E. C. Weinbach was elected to membership in the American Society of Biological Chemists.

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A. Project Description Sheet

1. NIAID-80
SERIAL NUMBER

2. National Institute of Allergy and Infectious Diseases
INSTITUTE OR DIVISION

3. Laboratory of Tropical Diseases
LABORATORY, BRANCH or DEPARTMENT

4. Section on Immunology
SECTION OR SERVICE

5.
LOCATION (IF OTHER THAN BETHESDA

6. Studies on the Mechanism of Allergy and Immunologic Phenomenon in Parasitic Diseases
PROJECT TITLE

7. John Bozicevich
PRINCIPAL INVESTIGATOR

8. Dr. J. E. Tobie, Mr. R. M. Pennington
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION:

Project Subtitle:

Study of Immune Response in Parasitic Diseases

Objectives:

To study by chemical and physical methods fractions of parasite extracts for use in immunologic diagnosis of parasitic diseases. To study the mechanism of antibody formation by histologic studies; to study the electrophoretic patterns of sera after immunization with specific fractions of antigen and by specific infection; to fractionate the antisera to determine the best fraction for immunization.

Methods Employed:

1. Antibodies will be tagged with radioactive isotopes and electro-

phoretic patterns will be developed by the agar diffusion method.

2. Antigens will be fractionated by electrophoresis in starch and agar and by eluting for specific antigenic fractions. Continuous electrophoresis, after Durum, will be employed for fractionation of antigen and anti-serum.

Major Findings:

It has been found that anti-kidney rabbit serum when labelled with radioactive iodine is specifically taken up in the rat kidney. The ovary and spleen also take up considerable antibody but not to the extent that the kidney removes the specific antibody.

Significance to the Program of the Institute:

Specific parasite anti-serum labelled with radioactive substances might be employed as a therapeutic agent, particularly where no chemotherapy has proven effective, such as echinococcosis and trichinosis.

Proposed Course of Project:

Labelling of antigens and antibodies will be conducted, and localization in vitro and in vivo will be employed to study the effect on infected animals and their serology. By electrophoretic methods it is hoped that concentration of the most effective fraction will be obtained.

Project Subtitle:

Studies on the Role of Polyglucoses as an Antibody Engender

Objectives:

To determine if high molecular polyglucoses are able to elicit specific antibody response in animals.

Methods Employed:

To prepare, chemically, high molecular polymers and to test these polymers by injecting into animals.

Major Findings:

The high molecular polyglucoses tried thus far have not shown immunologic response; that is, they are non-antigenic and antibodies could not be revealed.

Significance to the Program of the Institute

A number of synthetic blood plasma expanders have been tried without satisfactory results. It is possible that some of this may be explained by immunologic phenomenona.

Proposed Course of Project:

To continue preparation of higher molecular weight polyglucoses and to tag some of these for immunologic study.

Project Subtitle:

Studies on the Role of Accessory Factor in the Performance of the Dye test for Toxoplasmosis.

Objectives:

Attempts were made to isolate the factor from human plasma which contains the "accessory factor" employed in the dye test.

Methods Employed:

Plasma was fractionated by employing salt and ethanol. Adsorption on "Zymosan" was also employed.

Major Findings:

To date no satisfactory method of preparation of the accessory factor has been discovered.

Significance to the Program of the Institute:

Accessory factor is difficult to obtain for the diagnostic dye test for toxoplasmosis. If it could be obtained from blood plasma, a readily available and economical source of this material for the performance of the dye test would be at hand.

Proposed Course of Project:

This project will be dropped.

Project Subtitle:

Study of the Role of Serotonin and Histamine in Allergic Responses

Objectives:

To determine the components of parasite extracts responsible for increase in histamine and serotonin in immunized animals.

Methods Employed

Animals are immunized with various fractions of parasitic as well as biological materials. Tests for the detection of serotonin and histamine will be conducted *in vitro* and *in vivo*. Animals will be rendered

hypersensitive to dust from parasitic extracts and the animals will be subjected to "micro-shock".

Major Findings:

Studies showed that serotonin and histamine, as determined chemically, were liberated from the blood platelets when the specific antigen was added to the blood obtained from sensitized rabbits. In vivo, an immediate rise in plasma serotonin and histamine was noted following injection of a specific antigen into sensitized rabbits. The high level of serotonin was reached in one minute and at the end of two minutes the serotonin level returned to normal. The histamine level rose to a higher value and the return to normal was slower.

Significance to the Program of the Institute:

Since the findings of serotonin are parallel to those of histamine it is suggested that serotonin as well as histamine may play a role in explaining allergic manifestations.

Proposed Course of Project:

During the coming year a number of different species of animals will be studied since it has been found that the lungs of certain animals contain very little serotonin, if any, while others contain a great deal. It will be necessary to study the tissues of these animals in the normal and sensitized state. The serotonin level in the brain of these animals will also be studied.

Project Subtitle:

Fluorescent Antibody Studies on the Cellular Localization of Antigens and Antibodies in Parasitic Diseases

Objectives:

To obtain basic and practical information on the nature of immune responses including the cellular localization of antigen and the site of antibody formation in diseases of parasitic origin.

Methods Employed:

Initially, the conventional fluorescent antibody labelling technique will be employed. In order to study an antigen-antibody system of low order, attempts will be made to introduce a second system which is of a high order and thereby specifically localizing by an indirect method. The cells involved in antibody formation will be studied by a technique of "layering" in which untagged antigen is utilized to bind the antibody. Then by applying fluorescein-tagged antibody it will be possible to determine the localization and site of antibody formation in any particular disease.

Major Findings:

Localization of antigen in animals infected with Leptospira icterohaemorrhagiae has been studied by the fluorescent antibody technique. In the early stage of the disease, antigen was not detectable in the liver, but in the later stage it has been possible to visualize the antigen by its specific fluorescence. The antigen was of a particulate nature and was observed to involve principally the cytoplasm rather than the nucleus of certain cells. As judged by fluorescence microscopy it is believed that the majority of the cells so involved are the Kupffer cells. Positive identification as yet has not been accomplished due to the technical difficulties involved in the hematoxylin and eosin staining of fresh, frozen tissues after application of fluorescein-labelled antibody. It is believed that this difficulty will be resolved by the observation that tissues frozen by the conventional techniques show many ice crystal artifacts with a consequent loss of architecture, whereas if very rapid freezing in petroleum ether at -70°C. or in isopentane at -195°C. is used, the ice crystal artifacts are submicroscopic in size with preservation of the cellular architecture. Several of the significant findings have stemmed from the many technical difficulties entailed in the fluorescent antibody technique. One of these was the discovery that by exciting the labelled tissues at a maximum wavelength of 410-420 mu rather than at 356 mu, as is done in the technique of Coons, much greater fluorescence is produced. As a consequence the cellular elements are more clearly visible and the architectural orientation can be more easily observed. Furthermore, by using this method of excitation photographs have been taken which show considerably more than what has been observed in publications dealing with the fluorescent antibody technique.

Significance to the Program of the Institute:

The basic information obtained on cellular localization of antigen may lead to more intelligent investigation of chemotherapeutic agents. Furthermore, if the fluorescent antibody technique can be utilized as a diagnostic tool for earlier, specific detection of disease, it will aid the clinician in therapeutic management.

Proposed Course of Project:

The investigations concerned with the cellular localization of antigen in the various tissues will be completed in experimental leptospirosis. It would appear that the fluorescent antibody technique is quite a versatile tool and that a considerable amount of both basic and practical information can be obtained by expanding the studies to include other diseases of parasitic, bacterial, viral, or allergic origin. During the antigenic phase of certain diseases before antibodies appear, it seems that a serological-fluorescent antibody test could be devised for earlier specific diagnosis. In cases of doubtful serology or clinical diagnosis, the application of the technique to biopsy material could be very helpful. The nature of immune responses in the germ-free animal as compared to the conventional animal and the elucidation of the role of the reticulo-endothelial system in antibody formation can be studied by the fluorescent antibody technique.

Form No. OR-1
October 1956
(Attachment I)

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. PHAID-80
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$49,860	34,810	84,670	2.0	6.0	8.0

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2	7	9	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

(Use reverse and additional pages, if necessary)

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES of HEALTH

INDIVIDUAL PROJECT REPORT

Part C. Honors, Awards and Publications. 15. NIAID-80
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

Spicknall,C.G. Bozicevich, J.; Black,R.L; and Terry, L.L.: The complement fixation test in the diagnosis of amebiasis. Am. J. Gastroent. (In Press)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

ROCKY MOUNTAIN LABORATORY

Annual Report of Program Activities--Calendar Year 1956

NIAID-86	Office of the Director
NIAID-87	Transmission of Disease Agents by Certain Vectors
NIAID-88	Studies on Diseases Having a Reservoir of Infection in the Natural Environment
NIAID-89	The Relationship Between Viruses and Tumors
NIAID-90	The Allergy and Immunology of Infections Due to Histoplasma capsulatum
NIAID-91	The Mechanism of Delayed Allergy
NIAID-92	Morphologic Changes in Tissue Cultures of Mammalian Cells Infected with Certain Viruses
NIAID-93	Metabolic Studies of Tissue Cultures of Mammalian Cells Infected with Certain Viruses
NIAID-94	Metabolic Studies of Rickettsial Infections
NIAID-95	Immune Prophylaxis of Salmonella Infections: Separation and Immunologic Significance of Antigens
NIAID-96	Immune Prophylaxis of Mycobacterial Infections
NIAID-97	The Isolation of the Morphologic Structures of Mammalian Cells
NIAID-98	The Growth Characteristics of Varicella and Zoster Viruses in Tissue Culture
NIAID-99	Studies on Poliomyelitis with Special Emphasis on Certain Aspects of Vaccination
NIAID-100	The Community Health Project
NIAID-101	Studies on Colorado Tick Fever
NIAID-102	Studies on the Encephalitides

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-86
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. Office of the Director
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. None
PROJECT TITLE

7. Dr. Carl L. Larson, Director
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

The over-all direction and supervision of the scientific research program of the Rocky Mountain Laboratory; the direction of the administrative aspects of the Laboratory including all fiscal, supply, housekeeping and maintenance activities; and the providing of scientific services as required by the research staff, including laboratory animal care and breeding, sterile glassware and media preparation, special shop services, library, graphic arts, etc.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data

11. NIAID-86
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$81,500.		$81,500.	.50	14.75	15.25

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	.50	15.75	16.25	

13. BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☒
REVIEW & APPROVAL	☐	PROFESSIONAL & TECHNICAL ASSISTANCE	☐
BIOLOGICS STANDARDS	☐		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-86
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-87
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Transmission of Disease Agents by Certain Vectors
PROJECT TITLE

7. Cornelius B. Philip
PRINCIPAL INVESTIGATOR

8. Gordon E. Davis, Willy Burgdorfer, David B. Lackman, J. Frederick Bell, William J. Hadlow, James M. Brennan, and Glen M. Kohls
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The objectives of this program are to determine: (1) the efficiency of certain vectors in the transmission of disease agents, (2) the influence of certain factors upon the ability of vectors to serve as transmitting agents, and (3) the mechanisms involved in the growth of agents in certain invertebrate hosts.

Most of the methods employed have been described previously with the exception of an artificial feeding technique that has been developed to infect ixodid ticks with standardized amounts of micro-organisms. A fine capillary tube, which has been filled with infectious material by capillary action, is fitted over the mouth parts of the ticks. This allows for quantitative studies which cannot be carried out under conditions where animals are employed to produce infection in the ticks.

Major Findings:

Larvae of Aëdes aegypti that have been exposed to plasma from rhesus monkeys infected with yellow fever virus yield infected adult mosquitoes if the titer of the plasma is at least 10^4 mouse LD_{50}'s per 0.5 ml. In one experiment, 62% of the adult mosquitoes reared were infected with yellow fever virus. Transmission of yellow fever virus by Culex tarsalis was not successful.

It was demonstrated that trout infected with the organism responsible for salmon poisoning disease of dogs were not infectious for man. One animal continued to have sporadic febrile periods associated with rickettsiemia as long as 65 days after being infected.

When a method of artificial feeding was employed, it was found that Dermacentor andersoni and Amblyomma maculatum will feed readily at room temperature and will ingest 0.01 to 0.3 ml of fluid during a feeding period of 4 to 6 hours. The ticks do not feed to complete engorgement. Infections with rabies virus and various leptospirae were established in ticks by this method.

Various soft-shelled ticks were infected with the virus of Colorado tick fever. Virus could be detected in Ornithodoros parkeri and O. turicata only as long as 10 days following ingestion of infectious blood, while in O. nicollei, virus persisted until ecdysis occurred in 20 to 40 days.

Western equine encephalitis virus has been studied in a number of soft-shelled ticks. In O. moubata and O. turicata the virus is maintained for at least 30 days and is carried over from the early to the late nymphal stages. In O. nicollei, however, the virus disappears during ecdysis.

A method for counting leptospirae employing the congo red negative stain has been devised.

Significance to the Program of the Institute:

Much of the significance of this program is related to the interests of the group at Fort Detrick. The method of feeding ticks with capillary tubes makes it possible to study the mechanisms of infection of vectors in a quantitative manner. Great emphasis has

been placed upon the role of mosquitoes and hard-shelled ticks in the transmission of encephalitis and of Colorado tick fever. A knowledge of other vectors that may serve to maintain these diseases in nature would be of value in understanding the ecology of these diseases and eventually may be significant in developing control methods.

Proposed Course of Project:

Studies will be continued to determine if the rate of infection with yellow fever virus in adult mosquitoes may be increased when larvae maintained in infectious plasma are exposed to variables other than those already examined.

Studies on the behavior of the encephalitis viruses in hematophagous arthropods will be continued to determine those species that might be of significance in the maintenance and dissemination of these viruses in nature. This will be a combined field and laboratory investigation.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-87
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$86,500.		$86,500.	3.45	10.75	14.20
	($25,000.)		($25,000.)	(1.00)	(3.00)	(4.00)

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3.45	11.25	14.70	
	(1.00)	(3.00)	(4.00)	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Figures in parentheses represent contract work for Fort Detrick.

Form No. CRP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-37
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Davis, Gordon E.: A relapsing fever spirochete, Borrelia mazzottii (sp. nov.), from Ornithodoros talaje from Mexico. Am. J. Hyg. 63(1): 13-17. Jan. 1956.

2. Davis, Gordon E. and Mavros, Anthony J.: Observations on the biology of Ornithodoros d. delanoei Roubaud and Colas-Belcour, 1931 (Ixodoidea, Argasidae). Bull. Soc. Path. exot. 48(5): 698-704. 1955.

3. Burgdorfer, Willy and Owen, Cora Rust: Experimental studies on argasid ticks as possible vectors of tularemia. J. Inf. Dis. 98(1): 67-74. Jan.-Feb. 1956.

4. Philip, Cornelius B.: Comments on the classification of the Order Rickettsiales. Can. J. Microbiol. 2(3): 261-270. May 1956.

5. Davis, Gordon E.: World distribution of spirochetal diseases. 2. Relapsing fevers, louse-borne and tick-borne. (American Geographical Society, Plate 16) (Book Review). Am. J. Trop. Med. & Hyg. 5(2): 343-349. Mar. 1956.

6. Philip, Cornelius B.: Changes in the classification of Order Rickettsiales as proposed for 7th edition of Bergey's Manual. Part 2 of Symposium on Taxonomy by Robert S. Breed and others. Bact. Reviews 19(4): 271. Dec. 1955.

7. Davis, Gordon E.: The identification of spirochetes from human cases of relapsing fever by xenodiagnosis with comments on local specificity of tick vectors. Exp. Parasit. 5(3): 271-275. May 1956.

8. Davis, Gordon E. and Hoogstraal, Harry: Etude sur la biologie du spirochete Borrelia persica, trouve chez la tique Ornithodorus tholozani (Argasinae) recoltee dans le Governorate du desert occidental Egyptien - Commentaires sur la distribution et l'ecologie de la tique vectrice. Ann. de Parasit. Humaine et Comparee XXX(1-2): 147-154. 1956.

9. Kohls, Glen M.: Concerning the identity of Amblyomma maculatum, A. tigrinum, A. triste, and A. ovatum of Koch, 1844 (Acarina, Ixodidae). Proc. Ent. Soc. Wash. 58: 143-147. June 1956.

10. Davis, Gordon E. and Mavros, Anthony J.: An atypical Ornithodoros hermsi from Utah (Ixodoidea, Argasidae). J. Parasit. 42(3): 293-296. June 1956.

11. Kohls, Glen M.: The identity of Ixodes boliviensis Neumann 1904 and I. bicornis Neumann 1906. Proc. Ent. Soc. Wash. 58(4): 232-233. Aug. 1956.

12. Philip, Cornelius B. and Fairchild, G. B.: American biting flies of the genera Chrysotabanus Lutz and Cryptotylus Lutz (Diptera, Tabanidae). Ann. Ent. Soc. Am. 49(4): 313-324. July 1956.

13. Brennan, James M. and Beck, D. Elden: The chiggers of Utah (Acarina: Trombiculidae). Great Basin Nat. 15(1-4): 1-26. Dec. 31, 1955.

14. Fairchild, G. B., Philip, Cornelius B., Mackerras, I. M., and Oldroyd, H.: Proposed use of the plenary powers to validate the generic names Blaphella Bezzi, 1913 (Class Insecta, Order Diptera) and Lophiurethrum Gervais, 1850 (Class Mammalia). Bull. Zool. Nomenc. [illegible]: 195-199. Aug. 1956.

15. Davis, Gordon E. and Mavros, Anthony J.: Concerning the life cycle of Ornithodoros tholozani (Canestrini, 1890) (Ixodoidea, Argasidae). J. Egyptian Pub. Health Assoc. XXXI(2): 69-74. 1956.

16. Davis, Gordon E. and Mavros, Anthony J.: Observations on the biology of Ornithodoros foleyi Parrot, 1928 (Ixodoidea, Argasidae). J. Egyptian Pub. Health Assoc. XXXI(2): 65-68. 1956.

In press

1. Kohls, Glen M.: Eight new species of Ixodes from Central and South America (Acarina, Ixodidae). J. Parasit.

2. Brennan, James M. Neoschöngastia mouchetti, n. sp. and N. brennani Crossley and Loomis from Africa (Acarina: Trombiculidae). J. Parasit.

3. Hoyer, Bill H. A procedure for counting leptospiras based upon the congo red negative stain. J. Bact.

4. Kohls, Glen M. Ticks (Ixodoidea) of Borneo and Malaya. Malaysian Parasites. Published by the Institute for Medical Research, Kuala Lumpur, Malaya.

5. Kohls, Glen M. Ticks (Ixodoidea) of Micronesia. Insects of Micronesia. Published by Bernice P. Bishop Museum, Honolulu.

6. Jameson, E. W. Jr. and Brennan, James M.: An environmental analysis of some ectoparasites of small, forest mammals in the Sierra Nevada, California. Ecol. Mon.

7. Davis, Gordon E. and Mavros, Anthony J.: Observations on the biology of Argas brumpti Neumann, 1907 (Ixodoidea: Argasidae). Bull. Soc. Ent. d'Egypte.

8. Philip, Cornelius B.: New records of Tabanidae (Diptera) in the Antilles. Am. Mus. Novitates.

9. Philip, Cornelius B.: Records of horseflies in northeast Asia (Diptera, Tabanidae). Jap. J. San. Zool.

10. Philip, Cornelius B.: Descriptions of new Neotropical Tabanidae (Diptera) in the California Academy of Sciences. Pan. Pac. Ent.

11. Philip, Cornelius B.: Chapter "Class Microtatobiotes Philip." 7th edition of Bergey's Manual of Det. Bact.

12. Philip, Cornelius B., Hughes, Lyndahl E., and Darrow, D. I.: Experimental transmission of yellow fever virus by oriental mosquitoes. Proc. 10th Intern. Cong. Ent.

13. Philip, Cornelius B.: A helminth replaces the usual arthropod as vector of a rickettsialike disease. Proc. 10th Intern. Cong. Ent.

14. Larson, Carl L.: Tularemia. 4th edition of Diagnostic Procedures and Reagents, edited by Dr. William B. Cherry.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Philip, Cornelius B.

Appointed as Research Associate in the Department of Insects and Spiders of the American Museum of Natural History, for a period of 3 years, effective April 19, 1956.

Invited to give an address on "factors in the epidemiology of spotted fever before the Pacific Coast Branch of the American Entomological Society, Berkeley, California, June 27, 1956, for the symposium on Arthropod Transmission of Disease Pathogens.

Dr. R. E. Buchanan, chairman of the Judicial Commission of the International Committee of Bacteriological Nomenclature, requested collaboration in checking drafts of the revision of the International Code of Bacteriological Nomenclature.

Requested by Committee on Handbook of Biological Data, National Academy of Sciences, to prepare table and data on "Nomenclature and Systematic Criteria for Pathogenic Rickettsiae of Man," and to review tables on "Bacterial and Rickettsial Parasites of Man and Domestic Animals."

Requested to make a short tape recording on mosquito-borne encephalitides for the radio educational program during the meetings of the American Institute of Biological Sciences, August 28, 1956, Storrs, Connecticut.

Elected to the Executive Committee, Pacific Branch of the Entomological Society of America.

Presided as Chairman, Section D (Medical and Veterinary Entomology), Entomological Society of America, New York, December 27-30, 1956.

Kohls, Glen M.

Invited to participate in a "scientific working party" with scientists from England, Rhodesia, Australia, Egypt, Kenya, and the Union of South Africa for the purpose of studying ticks and arthropod-borne diseases for a period of about 2 months. This study is sponsored jointly by the East African Veterinary Research Organization and the Naval Medical Research Unit. No. 3 of Cairo, Egypt.

Lackman, David B.

Invited to present departmental seminar, Section of Preventive Medicine, Yale University School of Medicine, February 21, 1956, with the subjects, "The rickettsiae of the Rocky Mountain spotted fever group" and "Recent developments in the psittacosis picture."

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-88
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Diseases Having a Reservoir of Infection in the Natural Environment
PROJECT TITLE

7. J. Frederick Bell
PRINCIPAL INVESTIGATOR

8. David B. Lackman, William L. Jellison, William J. Hadlow, Lauri Luoto, Cora R. Owen, and Herbert G. Stoenner
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

Continued surveillance to determine the circumstances under which sporadic cases or an increased incidence of cases of such diseases as tularemia, Rocky Mountain spotted fever, leptospirosis, and rabies occur is an important adjunct to other studies concerned with the encephalitides and Colorado tick fever. In addition, the importance of Q fever as a public health problem is stressed, and methods of diagnosis and control are being sought.

The methods employed are well established ones, including both field and laboratory investigations.

Major Findings:

In a group of 37 bats originating in the Bitter Root area, only one was found to be infected with rabies virus. It is of interest that at the time of collection this was the only bat that was recognized as being ill.

Serological diagnoses of leptospirosis caused by Leptospira pomona and L. icterohaemorrhagiae were made in human beings.

The presence of Haplosporangium infection in rodents in Japan and Africa was demonstrated for the first time.

Through application of the capillary-tube agglutination test, Q fever infection was demonstrated in Ohio, Wisconsin, and Iowa.

Coccidioides immitis was again isolated from rodents in Washington County, Utah.

A ringworm infection in snowshoe hares caused by Trichophyton mentagrophytes was demonstrated. Because of the hares' mild reaction to the disease, they may serve as a reservoir for infection of other wild animals, domestic animals, or human beings.

Significance to the Program of the Institute:

The presence of rabies virus in bats is a problem of considerable concern to those interested in the control of this disease. With the addition of bats to the group of animals which are known to be infected in nature, the problems of control are multiplied. Systematic studies in this direction are urgently required.

The problem of Q fever is one which is extending rather than receding. The presence of the disease in dairy herds in Ohio, Wisconsin, and Iowa indicates that Q fever may become of significant public health importance in these States as it is already in California and Texas.

Proposed Course of Project:

While no expansion of this program is contemplated, it is felt that continued studies in this area are warranted, especially with rabies infections in bats and with Q fever infections in animals and man. The demonstration that vaccination of infected dairy herds will effectively eliminate Q fever infection among cattle suggests that this is an adequate method of control. The adaptation of the capillary-tube agglutination test to the diagnostic problems in Q fever has made epidemiological surveys in this field as practical as the application of the ring test to the study of brucellosis.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-88
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$39,300.		$39,300.	1.20	5.00	6.20
	($40,000.)		($40,000.)	(1.00)	(5.00)	(6.00)

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1.20	5.00	6.20	
	(1.00)	(5.00)	(6.00)	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Figures in parentheses represent contract work for Dugway Proving Grounds.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-88
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Stoenner, Herbert G.: Application of serology to the diagnosis of leptospirosis. Proc. Am. Vet. Med. Assoc. 172-176. 1955.

2. Adams, Lowell, Salvin, S. B., and Hadlow, W. J.: Ringworm in a population of snowshoe hares. J. Mammal. 37(1): 94-99. Feb. 1956.

3. Hadlow, W. J. and Grimes, Edward F.: Influence of stilbestrol-contaminated feed on reproduction in a colony of mice. Proc. Animal Care Panel 6: 19-25. 1955.

4. Owen, Cora R. and Larson, Carl L.: Studies on resistance to bacterial infections in animals infected with rickettsiae. J. Exp. Med. 103(6): 753-763. June 1956.

5. Jellison, William L.: Haplomycosis in Sweden. Nord. Vet.-Med. 8: 504-506. 1956.

6. Jellison, William L.: On the nomenclature of Besnoitia besnoiti, a protozoan parasite. Ann. N. Y. Acad. Sci. 64, Art. 2, pp. 268-270. July 1956.

7. Jellison, William L., Fullerton, W. J., and Parker, Hazel: Transmission of the protozoan Besnoitia jellisoni by ingestion. Ann. N. Y. Acad. Sci. 64, Art. 2, pp. 271-274. July 1956.

8. Stoenner, Herbert G., Crews, Frank W., Crouse, A. E., Taschner, Leslie E., Johnson, Charles E., and Wohleb, Joseph: The epizootiology of bovine leptospirosis in Washington. JAVMA 129(6): 251-259. Sept. 1956.

9. Burgdorfer, Willy: The possible role to ticks as vectors of leptospirae. I. Transmission of Leptospira pomona by the Argasid tick, Ornithodoros turicata, and the persistence of this organism in its tissues. Exp. Parasit. 5(6): 571-579. Nov. 1956.

10. Luoto, Lauri: A capillary-tube test for antibody against Coxiella burnetii in human, guinea pig, and sheep sera. J. Immunol. 77(5): 294-298. Nov. 1956.

In press

1. Owen, Cora R. and Buker, Emery O.: Factors involved in the transmission of Pasteurella tularensis from inoculated animals to healthy cage mates. J. Inf. Dis.

2. Jellison, William L.: Sodoku. Rat-bite fever due to Spirillum minus Carter. 4th edition of Diagnostic Procedures and Reagents, edited by Dr. William B. Cherry.

3. Bell, J. Frederick, Hadlow, W. J., and Jellison, William L.: A survey of chiropteran rabies in western Montana. Pub. Health Rep.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Hadlow, William J.

Elected to membership in the American Association of Pathologists and Bacteriologists, April 25, 1956.

Asked to collaborate with Drs. J. R. M. Innes and L. Z. Saunders in preparing a manuscript for a new book, "Diseases of the Central Nervous System of Domesticated Animals and Comparisons with Human Neuropathology." Specific chapters assigned thus far include one on "Conditions Caused by Microbial Toxins, Chemical and Plant Poisons" and "Diseases of Muscles."

Lackman, David B.

Invited to attend symposium on psittacosis sponsored by the Department of Animal Pathology, Rutgers University, held in New York City, February 16-17, 1956.

Courtesy appointment as lecturer in immunology at Montana State University, Missoula, Montana.

Elected to nominating committee of the American Society of Professional Biologists.

Luoto, Lauri

Requested to act as consultant for the Texas State Department of Health as expert on diagnosis of Q fever and field studies leading to control.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-89
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. The Relationship between Viruses and Tumors
PROJECT TITLE

7. Lawrence Kilham
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The possible etiologic relationships of viruses to tumors was the objective of this specific problem during the short period in which it was activated at the Rocky Mountain Laboratory.

Methods Employed:

Tissue-culture methods were employed to grow fibroma virus from tumors on cottontail rabbits.

Major Findings:

Fibroma virus was propagated in tissue cultures on cottontail testes. Two lines of this virus were carried through 14 successive passages in tissue culture. Growth of virus was accompanied by the formation of acidophilic intracytoplasmic inclusions and by vacuoles containing a substance with a ground glass appearance.

Significance to the Program of the Institute:

Propagation of this virus in tissue culture offers a means of studying the nature of this tumor-inducing virus, its effect on living cells, and its transformation into strains of greater or lesser virulence.

Proposed Course of Project:

This project has been inactivated due to the transfer of the investigator to the Division of Biologics Standards.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-89
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	none		none	none	none	none

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	none	none	none	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-89
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Kilham, L., Mason, P., and Davies, J. N. P.: Pathogenesis of fatal encephalomyocarditis (EMC) virus infection in albino rats. Proc. Soc. Exp. Biol. & Med. 90(2): 383-387. Nov. 1955.

2. Kilham, Lawrence, Habermann, R. T., and Herman, C. M.: Jaundice and bilirubinemia as manifestations of canine distemper in raccoons and ferrets. Am. J. Vet. Res. 17(62): 144-148. Jan. 1956.

3. Kilham, Lawrence: Serological studies of canine distemper--complement fixation with spleen antigens. Am. J. Vet. Res. 17(64): 398-401. July 1956.

4. Kilham, Lawrence: Propagation of fibroma virus in tissue cultures of cottontail testes. Proc. Soc. Exp. Biol. & Med. 92(4): 734-742. Aug.-Sept. 1956.

5. Kilham, L., Mason, P., and Davies, J. N. P.: Host-virus relations in encephalomyocarditis (EMC) virus infections. I. Infections of wild rats. Am. J. Trop. Med. & Hyg. 5(4): 647-654. July 1956.

6. Kilham, L., Mason, P., and Davies, J. N. P.: Host-virus relations in encephalomyocarditis (EMC) virus infections. II. Myocarditis in mongooses. Am. J. Trop. Med. & Hyg. 5(4): 655-663. July 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet 1. NIAID-90
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. The Allergy and Immunology of Infections Due to Histoplasma capsulatum
PROJECT TITLE

7. Samuel B. Salvin
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The objective of this project is to determine what effect the allergenic components of an infecting pathogen have on resistance to chronic infectious diseases such as histoplasmosis and tuberculosis.

The methods employed include the use of infections in mice with Histoplasma capsulatum, the production of immunity with isolated fractions obtained from Histoplasma organisms, and the transfer of hypersensitivity with white cells and of protective immunity with serum.

Major Findings:

Mice made resistant to infection with H. capsulatum by exposure to a sublethal infection showed two responses, depending on the time interval allowed for this resistance to develop. If the interval is approximately 5 days, a high level of protection is manifested, while if the interval is in the neighborhood of 14 days, mice develop a chronic reaction which proceeds to a fatal termination. Mononuclear cells from mice resistant to Histoplasma when transferred to recipient animals produce a decreased susceptibility to infection in the recipient. This is apparently on the basis of an allergic mechanism.

Significance to the Program of the Institute:

The question of the relation of allergy to immunity in such diseases as histoplasmosis and tuberculosis has been a matter of debate for many years. Whether or not allergy is a component of protective immunity is unsettled. Obviously, methods of specific prevention will depend for their application upon a resolution of this problem.

Proposed Course of Project

Experiments to differentiate those cells or components of the host which produce increased resistance and those which produce hypersensitivity are being carried out. Certain components of the yeast phase of H. capsulatum are being studied under the assumption that a purified antigen can be obtained which will produce resistance only and containing no component leading to increased hypersensitivity.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID 90
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$31,400.		$31,400.	.50	4.00	4.50

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1.00	4.00	5.00	

13. BUDGET ACTIVITY:

RESEARCH	[X]	ADMINISTRATION	
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-90
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Salvin, S. B.: Acquired resistance in experimental histoplasmosis. Trans. N. Y. Acad. Sci. Series II, 18(5): 462-468. Mar. 1956.

2. Ribi, Edgar and Salvin, S. B.: Antigens from the yeast phase of Histoplasma capsulatum. I. Morphology of the cell as revealed by the electron microscope. Exp. Cell Res. 10(2): 394-404. Apr. 1956.

3. Grayston, J. Thomas and Salvin, S. B.: Experimental histoplasmosis in immunized and nonimmunized mice. Comparative pathology. AMA Arch. Path. 61(5): 422-433. May 1956.

4. Salvin, S. B.: Precipitin and complement-fixation tests with whole yeast phase antigen. Pub. Health Monograph No. 39 (PHS Pub. No. 465). 163-165. 1956.

5. Salvin, S. B.: Immunization of mice against Histoplasma capsulatum. Pub. Health Monograph No. 39 (PHS Publ. No. 465). 107-109. 1956.

6. Salvin, S. B.: Growth of Histoplasma capsulatum in liquid medium. Pub. Health Monograph No. 39 (PHS Publ. No. 465). 61. 1956.

7. Salvin, S. B. and Furcolow, M. L.: The precipitin test in human histoplasmosis. Pub. Health Monograph No. 39 (PHS Publ. No. 465). 129-131. 1956.

8. Salvin, S. B.: Discussion of serology of histoplasmosis. Pub. Health Monograph No. 39 (PHS Publ. No. 465). 46-47. 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-91
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. The Mechanism of Delayed Allergy
PROJECT TITLE

7. Samuel B. Salvin
PRINCIPAL INVESTIGATOR

8. E. John Bell
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

In spite of considerable research, the basic antigen-antibody mechanism responsible for the production of the delayed type of hypersensitivity remains obscure. The objectives of this study are: (1) to determine whether or not delayed hypersensitivity can be produced in the absence of circulating antibody after the injection

of an antigen-antibody complex, (2) to determine the temporal relations of the immediate and delayed types of hypersensitivity following the injection of a soluble antigen, and (3) to determine the role of cells from sensitized animals grown in tissue culture in the production of delayed allergy.

Methods Employed:

The methods employed are relatively standard except for the use of tissue-culture preparations of white cells derived from whole blood of sensitized and of normal guinea pigs.

Major Findings:

It has been found that delayed hypersensitivity can be produced in guinea pigs in the complete absence of circulating antibody after injection of an antigen-antibody complex prepared in the region of high antibody excess. When soluble antigen is administered to guinea pigs, the delayed type of allergy precedes the immediate type of allergy. When a dose of 1 Lf diphtheria toxoid is administered, no skin reactions occur for the first 3 days. On the fourth day the delayed type of allergy appears, but there is no detectable circulating antibody. This condition persists until the tenth day, at which time both circulating antibody and the immediate type of allergy can be demonstrated. An increase in the amount of antigen contained in the sensitizing dose decreases the above intervals, while a decrease in the sensitizing dose increases the time intervals.

Cells from sensitized guinea pigs grown on tissue cultures for at least 4 weeks have retained their ability to sensitize recipient guinea pigs.

Significance to the Program of the Institute:

The problems of allergy and immunity appear to be intimately related, and the intimacy of this relationship has made it extremely difficult to separate these conditions. If it is possible to produce any type of allergy in the complete absence of circulating antibodies, it will obviously be a much simpler matter to study the nature of allergy in such a system. The quantitative and temporal aspects of the production of delayed and immediate types of hypersensitivity will also aid in an understanding of the mechanisms involved. If it is possible to produce sensitized cells either by continuous growth of cells from a sensitized donor, or by sensitizing cells *in vitro* from a nonsensitized donor, an understanding of the role of cellular mechanisms in the production of allergic manifestations will be at hand.

The studies under way will be continued and expanded. Antigens labeled with radioactive iodine 131 will be injected into guinea pigs in an attempt to determine the course of development of the delayed type of allergy. The differences in growth and metabolism in tissue culture of cells from normal and sensitized guinea pigs will be studied. Attempts will be made to produce allergy in normal cells growing in tissue cultures and to determine the response to homologous serum of sensitized cells grown in tissue culture. Attempts will be made to produce sufficiently large quantities of sensitized cells in tissue culture to make it possible to study and isolate the cell structures responsible for the production of allergic phenomenon.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-91
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$47,100.		$47,100.	1.00	6.00	7.00

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1.50	6.00	7.50	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-91
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Uhr, J. W., Salvin, S. B., and Pappenheimer, A. M.: Production of delayed sensitivity to protein without detectable circulating antibody. Fed. Proc. 15(1): 619. Mar. 1956.

In press

1. Uhr, Jonathan W., Salvin S. B., and Pappenheimer, A. M. Jr.: Delayed hypersensitivity. II. Induction of hypersensitivity in guinea pigs by means of antigen-antibody complexes. J. Exp. Med.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-92
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Morphologic Changes in Tissue Cultures of Mammalian Cells Infected with Certain Viruses
PROJECT TITLE

7. Bill H. Hoyer
PRINCIPAL INVESTIGATOR

8. Nick J. Kramis, Daniel B. Ritter, and E. John Bell
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The objectives are to study the gross morphologic reactions of tissue-culture cells to various viruses in order to better understand the sequence of changes that cells undergo when infected with viruses and to compare the reaction of cells to various viruses (and virus dosages) for the determination of possible similarities and dissimilarities in the systems employed.

Methods Employed:

Phase contrast time-lapse photocinemicrography was used to follow the changes which took place in monkey-kidney, HeLa and the Detroit series of cells infected with various viruses.

The effect of various dosages of virus was studied by placing inocula which contained about 1000 Saukett polio $TCID_{50}$ per cell to 1 $TCID_{50}$ per cell in an overlay and subjecting monkey-kidney cells to this overlay under standardized conditions. Infected cells were then photographed, using standardized conditions.

The Detroit series of cells was subjected to a standardized inoculum of Saukett poliovirus in Eagle's medium, and the individual reactions of each of these cells and of the HeLa cell were observed in a series of time-lapse motion pictures.

Major Findings:

When exposed to high dosages of Saukett poliovirus, monkey-kidney cells become round in 3 to 4 hours as opposed to 6 to 8 hours under similar conditions with low dosages. With low dosages, monkey-kidney cells become round, bubble, and then retract the bubbles and become round again. With high dosages, the cells in many instances seem to become round without going through the preliminary stages.

The Detroit series of human epithelial-like cells and the HeLa cells all react very similarly to the Saukett poliovirus under standardized conditions.

Some of the cell reactions that precede giant cell formation in virus-infected monkey-kidney cells have been photographed.

Significance to the Program of the Institute:

A consideration of the sequence of morphologic effects which occur among various lines of tissue-culture cells following infection with different viral agents yields knowledge that may be of significance to interpretations of methods of viral reproduction.

Proposed Course of Project:

Studies of morphologic changes of virus-infected cells will be continued in order to include a wider range of such changes in appropriate motion pictures. New equipment will make possible studies of those viruses which take a considerable length of time to kill the cells.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-92
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$23,500.		$23,500.	.75	3.00	3.75

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1.00	3.00	4.00	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-92
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

In press

1. Kramis, N. J.: Time-lapse, phase contrast cine photomicrography of tissue culture cells. J. Biol. Photo. Assoc.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Hoyer, Bill H.

Courtesy appointment as lecturer in bacteriology at Montana State University, Missoula, Montana.

Kramis, N. J. and Hoyer, Bill H.: "Gross Morphologic Changes of Virus-Infected Cells." Motion picture, produced in 1956. Available through Rocky Mountain Laboratory and The Committee on Visual Education of the Society of American Bacteriologists.

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-93
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Metabolic Studies of Tissue Cultures of Mammalian Cells Infected with Certain Viruses
PROJECT TITLE

7. Bill H. Hoyer
PRINCIPAL INVESTIGATOR

8. Daniel B. Ritter
OTHER INVESTIGATOR

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The objectives are to study the formation of amino acids and nucleic acids by both normal and virus-infected tissue-culture cells.

Cells are fed limiting amounts of such tracers as randomly labeled C^{14} glucose or P^{32}-containing orthophosphate. At various intervals during the experiment, samples are withdrawn and the sequence of formation of various compounds in both normal and infected cells is determined by a combination of chromatography and radioautography. In some experiments the tracer is added to normal and infected cells for a specified interval during the course of the experiment in order to isolate the reactions taking place during that interval.

In addition, HeLa cells have been fed various C^{14}-amino acids which have been determined essential by Eagle. By chromatography and radioautography the incorporation of C^{14} from these amino acids into large molecules of the cell was studied.

Major Findings:

The series of human epithelial-like cells react similarly to infections with viral agents, and it was reasonable to suppose that they would show similar metabolic reactions. It was determined that all of the Detroit cells (8) and the HeLa cells incorporated C^{14} from a randomly labeled glucose-fructose mixture into aspartic and glutamic acids, alanine, and valine, which were found in acid hydrolysates of proteins of these cells. It is also interesting to note that although valine is a required amino acid, these cells can produce some valine from components of C^{14} sugars.

Methionine, valine, leucine, isoleucine, and phenylalanine are among those amino acids considered to be essential for the growth of the HeLa cell. When these C^{14}-labeled amino acids are added to Eagle's medium modified to be limiting for one or a group of these amino acids, radioactivity is found in the particular amino acid or group of amino acids only.

With the cooperation of Ellis Bolton, of the Carnegie Institution of Washington, it was determined that orthophosphate may be utilized as such by mammalian cells in tissue culture. Conditions for making reproducible runs in which $P^{32}O_4$ incorporation is followed have been determined, and the sequence of appearance of nucleic acid precursors has been observed. Although observations are preliminary, it appears that incorporation of $P^{32}O_4$ into adenine phosphates is severely impaired by virus infection and that infected cells retain predominantly guanine phosphates in their alcohol-soluble fractions. Cells which have been fed $P^{32}O_4$ have been infected with vaccinia virus and in replicate series it appears that about 0.1% of the $P^{32}O_4$ bound in the Detroit 98 cell appears in the virus-containing fraction which has been determined to be relatively clear by electron microscopy.

The results obtained indicate that the reactions of the cells under consideration are similar in respect to both virus infection and their ability to incorporate glucose into aspartic and glutamic acids, alanine, and valine. The fact that valine can be produced from such sugars indicates that it is not required in the sense that certain other amino acids are needed by tissue culture cells. Thus methionine, valine, leucine, isoleucine, and phenylalanine are required amino acids which are used as such by the cells and are not converted into other amino acids or carbon-containing compounds.

The similarity between the nucleic acid cycle in bacteria and in mammalian cells in tissue culture is striking. It would appear from the results obtained that this process is basically the same throughout a wide range of living organisms. The fact that cells retain guanine phosphates and are unable to produce adenine phosphates when infected by viral agents may be of significance in an understanding of the mechanisms of virus reproduction.

Proposed Course of Project.

Effects of various viruses on the nucleic acid metabolism of tissue-culture cells will be studied more intensively, particularly from the standpoint of changes occurring in the formation and incorporation of $P^{32}O_4$ labeled precursors into nucleoprotein fractions during normal and virus influenced periods. In conjunction with the above studies, efforts will be made to devise simple methods for making purified radioactive viruses so that refined serologic studies and infection studies in whole animals may be done.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-93
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$23,000.		$23,000.	.50	3.00	3.50

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	.75	3.00	3.75	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-93
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-94
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Metabolic Studies of Rickettsial Infections
PROJECT TITLE

7. Richard A. Ormsbee
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The objectives are to elucidate some aspects of rickettsial metabolism and of cells infected with rickettsiae and to determine the metabolic relationship existing between parasite and host cell.

Methods Employed:

Rickettsiae will be grown in tissue culture and the metabolic consequences will be followed with the aid of radioisotopes, chromatography, and other conventional biochemical procedures.

Major Findings:

This project has just been initiated.

Significance to the Program of the Institute:

The problems associated with infection of cells with agents which are obligate intracellular parasites are extremely complicated. This is especially true when the infective agent under consideration is a virus. The possibilities of detecting metabolic activity of such agents are remote, whereas with such organisms as rickettsiae there is reasonable opportunity to determine the contributions of host and the agent to the total metabolic process of the infectious system. With such demonstrations of the effects upon amino acid metabolism and nucleic acid metabolism by viral agents on tissue-culture cells as have been made in this laboratory, it would seem probable that these techniques will yield pertinent information in this specific area.

Proposed Course of Project:

The proposed course of the project has been generally outlined under "Methods."

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-94
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$39,000.		$39,000.	1.00	5.00	6.00

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1.00	5.00	6.00	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, and Publications 15. NIAID-94
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Ormsbee, Richard A.

Invited to give a seminar in the Section of Preventive Medicine, Yale University School of Medicine, May 21, 1956, on "Orphan Viruses."

Courtesy appointment as lecturer in biochemistry at Montana State University, Missoula, Montana.

Appointed Research Fellow, Yale School of Medicine for 1954-56.

Invited to participate in New York Academy conference on "Viruses in Search of Disease."

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-95
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Immune Prophylaxis of Salmonella Infections: Separation and Immunologic Significance of Antigens
PROJECT TITLE

7. Edgar Ribi
PRINCIPAL INVESTIGATOR

8. Kelsey C. Milner and Bill H. Hoyer
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

To study the surface of microorganisms whereby components of the surface are isolated with retention of biologic activities and characterized to permit ultimate correlation of chemical composition and structure to protective, endotoxic, and sensitizing activities.

Methods Employed:

The study of antigenic constituents of the microbial agents is primarily dependent upon their separation from the cells by methods sufficiently mild that the material isolated is not significantly altered in the process. Exposure of cells to physical treatments, including bombardment with minute glass beads and osmotic shock, in combination with centrifugations and electron microscopy results in the separation of cell wall and internal protoplasm. Further separation such as the extraction of unaltered endotoxin with water from the cell walls of live Salmonella enteritidis is accomplished during removal of minor amounts of certain fats by shaking of water suspensions with ether. Purification procedures of isolated antigen, which include fractional precipitation with alcohol and salts, extraction with organic solvents, and other methods, are followed by means of optical ultracentrifugation, paper electrophoresis, paper chromatography, ultraviolet absorption, X-ray diffraction, and chemical analytical analysis (partly on radioactive labeled material). The fractions are tested both serologically and in animals for their behavior in immune reactions including protection, toxicity, and hypersensitivity.

Major Findings:

Previous progress reports dealt with the finding that call walls of organisms so far studied, such as Bacterium tularense, Histoplasma capsulatum, and Salmonella enteritidis, are the site of the essential antigens.

The toxin released by our method left the cell wall morphologically intact and capable of protecting animals against infection, indicating that endotoxin is not necessarily identical to the antigen eliciting the formation of protective antibodies. Because the crude toxic material also showed some protection, attempts to separate the remaining antigenic activity from the toxic activity were made. Sedimentation studies with the optical ultracentrifuge revealed that the crude toxic material, after it has been dialyzed, is a mixture of at least five components. Chemically, the fraction contains protein, polysaccharide and lipid. Subsequent fractional precipitation with alcohol of aqueous solutions containing varying concentrations of sodium chloride and fractional precipitation with ammonium sulfate resulted in toxic material essentially devoid of protein. The remaining fraction, consisting mainly of lipid and polysaccharide, behaved as a single molecular entity when studied in the ultracentrifuge. Purification of the crude toxin did not result in a change in the mouse lethal activity, but resulted in a twofold increase of the precipitation test titer.

Up to the present time, methods have not been reported which allowed the isolation of so-called bound lipid with retention of lethal activity from endotoxins (earlier methods included hydrolysis with dilute acids). We found that treatment with hot alcohol resulted in dissociation of lipid from the toxin complex. After such

treatment, lipid could be extracted in considerable amounts with common fat solvents such as ether. The material remained toxic after lipid extraction. It therefore would appear that toxic activity is correlated with a polysaccharide. Fractions of purified toxin have not yet been tested for protective value.

Significance to the Program of the Institute:

Further proof of the theory that the antigens producing protective antibodies are contained in the cell walls of microorganisms is produced by this study. The isolation of such an antigen dissociated from elements of toxicity should be helpful in producing vaccine against Salmonella infections in man.

It is felt that the results of separation of endotoxin into various fractions employing mild physical and chemical methods demonstrate that so-called complete antigens of Boivin are merely artifacts produced by treating organisms in a rigorous fashion.

Another general finding which has emerged from these investigations is the concept that in attempting to isolate antigens responsible for protection against infection, a biological system which involves an infectious process rather than a toxic reaction should be employed.

Proposed Course of the Project:

Studies will be continued on endotoxins from Salmonella sp. to permit ultimate correlation of chemical composition and structure to toxic activity and to permit testing of purified toxin for its protective value, and further, to provide a suitable method for isolation of the somatic antigen of the detoxified cell wall to permit correlation of chemical composition and structure to protective ability.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-95
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$94,200.		$94.200.	2.25	11.75	14.00

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3.25	12.00	15.25	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-95
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-96
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Immune Prophylaxis of Mycobacterial Infections
PROJECT TITLE

7. Edgar Ribi
PRINCIPAL INVESTIGATOR

8. Bill H. Hoyer, Cora R. Owen, J. Frederick Bell, and Carl L. Larson
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The objective of this program is to attempt to define morphological sites of antigenic materials which contribute to the immunologic response characteristics of infections produced by acid-fast bacilli.

The methods employed will be centered about the use of physical methods to detect and isolate antigenic fractions. The biological aspects will be studied by methods which are acceptable in this field.

Major Findings:

The problem of obtaining cell walls from mycobacteria is a difficult one because of the presence of waxes, etc., which cause the organisms to adhere to each other. The addition of surface-depressing agents has decreased this tendency and has made it possible for us to obtain pure cell walls and, incidentally, to solubilize the protoplasmic material. Cell walls have been obtained from Mycobacterium butyricum and from the BCG bacillus.

Significance to the Program of the Institute:

It is obvious that if a killed vaccine were available for the prevention of tuberculosis this would be a major accomplishment in the field of preventive medicine. In studies made in other laboratories, elements which appeared to be cell walls of mycobacteria were depicted in electron microphotographs of those preparations capable of immunizing experimental animals. The fact that we have been able to obtain cell walls indicates that it may be possible to proceed with an analysis of the antigenicity of mycobacteria without too much difficulty with the preliminary steps.

Proposed Course of Project:

Research in this area will be expanded. Toxicity of the fractions will be tested by all methods available at present, and immunity experiments will be set up in white mice and guinea pigs. Studies will be directed largely toward obtaining an immunogenic fraction which possesses as little allergenic potency as possible.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-96
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$62,800.		$62,800.	1.15	7.75	8.90

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	1.60	8.25	9.85	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-96
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Ribi, Edgar

Requested to supply electron microscope photographs of fungi to illustrate the book, "Mycologische Serodiagnostik" to be written by Dr. Seeliger, of the University of Bonn, Germany.

Requested to supply the Society of American Bacteriologists electron micrographs of bacteria and fungi for their visual instruction program in microbiology.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet 1. NIAID-97
SERIAL NUMBER

2. NIAID
INSTITUTE OF DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. The Isolation of the Morphologic Structures of Mammalian Cells
PROJECT TITLE

7. Edgar Ribi
PRINCIPAL INVESTIGATOR

8. Carl L. Larson and J. Frederick Bell
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

To separate by physical means the cytoplasmic, cytoplasm, nuclear membrane, and nuclear material of mammalian cells in order to permit studies of the chemical composition and properties of the isolated elements, such as virus absorption by the cytoplasmic walls and nuclear walls and localization of host defense mechanism.

Structural elements are separated by physical methods, including mechanical vibration, osmotic shock, and fractional centrifugation procedures. To be successful these procedures must be carefully controlled by light and electron microscopy.

Major Findings:

Preparations of empty cytoplasmic and nuclear membranes and dispersed internal material have been obtained. The cytoplasmic membrane is extremely thin and fragile whereas the nuclear membrane is relatively thick and rigid. However, fractions containing cytoplasmic membranes or nuclear membranes alone have not yet been obtained.

Significance to Program of the Institute:

These findings support those of Landy and Shear. From the standpoint of our laboratory, the preparation of cell walls from mammalian cells would make it feasible to perform studies on the mechanism of viral infections. With such isolated fractions it should be possible to determine many of the factors involved in the process of attachment of viruses to cells, which is the original phase of cell invasion.

Proposed Course of the Project:

This project was very recently initiated in order to obtain host polysaccharides from mammalian cell walls in studies related to nonspecific protection which are conducted by the polysaccharide group of the NCI (Drs. Landy and Shear). Studies of infectious agent-host relationship may be conducted with such separated structural elements of the host.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-97
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,500.		$15,500.	.25	2.00	2.25

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	.30	2.00	2.30	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-97
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-98
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. The Growth Characteristics of Varicella and Zoster Viruses in Tissue Culture
PROJECT TITLE

7. E. John Bell
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSWEHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The ultimate objects of this program are to develop methods for performing serological tests for the diagnosis of disease produced by varicella virus and to develop methods for the preparation and testing of vaccine prepared from this agent. In order to approach these practical problems, it is necessary to find ways of separating virus from living infected cells maintained in tissue culture or to cause infective virus to be released into the culture medium during growth.

Methods employed have had three general approaches: (1) determination of growth characteristics exhibited by the virus cultured in various cell types, (2) investigation of the effect of various physical, chemical, and biologic factors on the growth properties of the virus in tissue culture, and (3) attempts to adapt the virus to growth in chick embryo and in laboratory animals.

Major Findings:

Varicella virus has been grown on various cell types, e.g., HeLa, Maben, human foreskin, monkey kidney, and monkey testes. No cytopathic changes were observed in human gut or conjunctival cells, KB cells, or Osgood's leukemia cells. It has not been possible to obtain virus free in culture medium, and negative results have been obtained in preliminary attempts to release infective virus by treating infected cells by sonic vibration, molar NaCl, citric acid, or poliovirus.

A strain of virus isolated from a case of herpes zoster has been adapted to produce complete infection or degeneration of cells in from 3 to 6 days.

Significance to the Program of the Institute:

The results to date have not yielded information of particular value, but it is apparent that the virus-tissue-culture system in use presents a host-parasite relationship which differs from most others which are employed in the laboratory. Furthermore, since this is an important disease of children, studies with this virus are merited.

Proposed Course of Project:

The project will be continued along lines described above. Studies on the pathogenesis of herpes zoster infection are contemplated.

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PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-98
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$15,500.		$15,500.	.25	2.00	2.25

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	.25	2.00	2.25	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-93
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet 1. NIAID-99
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Poliomyelitis with Special Emphasis on Certain Aspects of Vaccination
PROJECT TITLE

7. Carl M. Eklund
PRINCIPAL INVESTIGATOR

8. Carl L. Larson, Robert K. Gerloff, and Mary Casey
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The studies which were started in the spring of 1955 are nearing completion and this project has been set up for continuation this year. The investigations center mainly on vaccination of children in Montana and Idaho and on the isolation of virus from vaccines when tested in cortisonized monkeys.

The methods employed are those that are generally accepted in this field. The monkey test is one which was devised in this laboratory.

Major Findings:

A continuation of the studies that resulted from the so-called "Cutter incident" in Idaho indicates that the spread of virus following the administration of viable vaccine was not great.

During the summer of 1954, 38% children were vaccinated in Montana with a commercial lot of vaccine. During 1956, 24% of these children were still under study. While the study has not been completed, it would appear that all those who were vaccinated in 1954 as part of the N.F.I.P. program and who received one booster dose of vaccine in 1955 have a significant level of antibodies. None were negative against type 1 poliovirus, 1% was negative against type 2, and 4% against type 3. Among those who received a single dose of vaccine in 1955 only, these figures are 37%, 13%, and 4%, respectively. A booster dose of vaccine was given to these individuals in 1956. The results indicate that the vaccine presently employed is not as efficient as that used in the field trials, since 28% have no antibodies against type 1, 18% have no antibodies against type 1 virus, although all children have antibodies against type 2 virus. During the course of this study, it has become apparent that there is a definite enhancing effect of pre-existing antibody upon the production of heterologous antibodies. Types 1 and 2 have a mutual enhancing effect, while type 3 enhances type 2 but not type 1.

In testing Cutter vaccine lot 6039 in rhesus, cynomolgus, and African green monkeys, it was found that the paralysis rate is somewhat greater in cynomolgus monkeys than in the other species and that paralysis resulting in rhesus monkeys following inoculation of viable vaccine is always due to type 1 poliovirus. Viremia occurs only after the onset of paralysis. The brown fat and lymph nodes serve as an excellent source for the isolation of poliovirus. Vaccine lot 6039 appears to contain about one 50% paralytic unit of each type of virus in the dose of vaccine ordinarily employed.

Significance to the Program of the Institute:

It is important to continue our studies of the Cutter incident in Idaho, since this will develop data on the actual inoculation of live virus in children and the effects of such a procedure upon the general population. This of course is a procedure which should not be repeated. The immunologic response of children to the injection of killed vaccine may well be different in areas such as Montana and Idaho than would be the response in areas of greater population density. This is especially to be considered because of the cross reactions that have been demonstrated in the present investigations.

Another lot of Cutter vaccine will be tested in monkeys to determine its infectivity and to compare these results with those obtained with lot 6039.

The serological studies on children vaccinated in this area should be completed this year, and this phase of the project will then be terminated.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-99
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$78,500.		$78,500.	3.10	9.75	12.85

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3.60	11.00	14.60	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-99
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

1. Eklund, Carl M. and Larson, Carl L.: Outbreak of type 3 poliomyelitis on St. Paul Island, Alaska. Am. J. Hyg. 63(2): 115-126. Mar. 1956.

2. Eklund, Carl M., Bell, E. John, and Hadlow, W. J.: Detection of live virus in certain lots of poliomyelitis vaccine by inoculation of monkeys. Am. J. Hyg. 64(1): 85-91. July 1956.

3. Technical Committee on Poliomyelitis Vaccine and Subcommittee on the Monkey Safety Test: The monkey safety test for poliomyelitis vaccine. Am. J. Hyg. 64(1): 104-137. July 1956.

4. Shannon, James and Larson, Carl L.: Poliomyelitis vaccine: Some considerations relating to its safety. Bull. N. Y. Acad. Med. 32(10): 734-746. Oct. 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-100
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Community Health Project
PROJECT TITLE

7. Lauri Luoto
PRINCIPAL INVESTIGATOR

8. Cora R. Owen, Mary Casey, Max T. McKee, William J. Hadlow, W. H. Gaub
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The objective of this program has been the determination of the causes of illness occurring within a rural community, with special emphasis on those of viral etiology. Since the conditions of spread of infectious diseases may be different in rural areas from those that influence spread in urban areas, it was felt that the importance of these diseases could be judged more aptly in a rural environment. A further objective was the study in detail of

such diseases as appeared to be most important, judging from the data which were accumulated by survey methods.

The development of methods for the isolation of agents and for the serological diagnosis of the prevalent diseases was also considered as part of the program.

Methods Employed:

The methods employed have been those most recently developed for the study of viral infections, with the additional application of such complement-fixation tests and neutralization tests as have been applied in this laboratory, and the use of a wide spectrum of tissue-culture cells for isolation of agents.

The study has a combined field and laboratory approach.

Major Findings:

Studies of the sewers in the Hamilton area have shown that the use of a number of tissue-culture cells greatly enhances the pick-up rate of viruses. Employing HeLa cells only, by and large, one does not encounter agents other than polioviruses, whereas if monkey-kidney cells and Maben cells are included, a wide spectrum of viral agents may be isolated. It has been possible to isolate from a single culture of monkey-kidney cells two or more types of poliovirus in addition to Echo or Coxsackie viruses.

An outbreak of respiratory illness occurring during the summer months was shown to be due to adenovirus type 4. Virus was isolated from swabs taken from the local swimming pools.

An outbreak of influenza due to A' virus was studied.

Methods have been developed for the serological diagnosis of Coxsackie virus infections, employing antigens prepared from suckling rabbits and antiserums prepared in rabbits from these antigens. Complement-fixation tests performed with this type of material indicate that the complement-fixation test with this group of viruses has the same relation to the neutralization test as in the case of the polioviruses.

The serological study of persons in various age groups in this community indicates that the rate of infection within each age group against A and B Coxsackie virus is similar to the rate of incidence of infection with poliovirus.

The incidence of streptococcal infections and the number of individuals harboring streptococci have been notable. A series of cases of nephritis associated with the isolation of Beta hemolytic streptococci occurred during the winter months.

In a survey of children in the Hamilton school system, it was found that among first graders 18.5% of 366 tests yielded streptococci, whereas among high school freshmen 9.8% of 988 tests yielded positive results. These were presumably healthy children.

Among individuals who were ill, 10.5% of all specimens yielded Beta hemolytic streptococci and, of these, 52% were found to be A12's.

A wide variety of cells grown in tissue culture have been adapted to growth on horse serum and these have been employed for isolation purposes.

The KB cell has been found to be the most sensitive cell for survey purposes.

Significance to the Program of the Institute:

Studies performed to date indicate that the population of a rural community is continuously being infected by a wide variety of agents. The agents isolated up to the present time do not appear to be materially different from those isolated during studies in other areas. The most frequent agent encountered appears to be Beta hemolytic streptococci. This parallels observations that the Rocky Mountain area has a relatively high incidence of rheumatic fever and nephritis.

It has been obvious from these studies that the techniques and methods presently available are sufficiently sensitive to enable us to isolate agents from only a relatively minor portion of individuals presenting febrile illnesses.

Proposed Course of Project:

It still appears that an epidemiological study of febrile illnesses in a rural community could contribute much to our knowledge of the spread of infectious diseases. However, since we have been unable to isolate virus from the majority of specimens from sick persons, we shall attempt to develop more satisfactory methods for the definition of these febrile illnesses.

Those factors influencing the sensitivity of the methods will be reviewed and studied and more sensitive tissue-culture systems will be sought for. Carefully selected and handled specimens from ill persons will serve as the source of material for this study.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-100
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$62,800.		$62,800.	2.70	7.75	10.45

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	3.70	8.25	11.95	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-100
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None

Form No. ORP-1

Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet

1. NIAID-101
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on Colorado Tick Fever
PROJECT TITLE

7. Carl M. Eklund
PRINCIPAL INVESTIGATOR

8. Glen M. Kohls, James M. Brennan, William L. Jellison, David B. Lackman, William J. Hadlow, and Willy Burgdorfer
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO.(S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

The importance of Colorado tick fever as a public health problem has not been determined. The clinical disease in certain instances is associated with severe encephalitis and in others with serious hemorrhagic phenomena. More data on the clinical aspects of the disease, together with age, sex, and regional distribution,

are needed. Studies are [illegible] made to define the geographic distribution of infected ticks and to obtain ecological data which would explain the tick cycle in nature and why the disease is confined to the western United States.

Investigations of methods for isolation of [illegible] and for serological tests for the disease are being carried on.

Methods Employed:

The methods employed have been detailed in [illegible] reports. The application of the complement-fixation test [illegible] methods and of tissue-culture techniques for the isolation [illegible] being made.

Major Findings:

Virus was isolated from [illegible] patients from [illegible] Colo. Montana, Nevada, Oregon, South Dakota, Utah, and Wyoming [illegible].

Although laboratory experiments have shown that [illegible] of Dermacentor variabilis can be infected by feeding [illegible] and that the virus persists into the nymphal and adult stage [illegible] was isolated from approximately 1,000 of these ticks [illegible] from Nebraska, Saskatchewan, New Jersey, and Rhode Island.

Colorado tick fever virus was isolated from [illegible] of Dermacentor andersoni nymphs collected from two spotted ground squirrels in the Bitter Root Valley. Antibodies have been demonstrated in ground squirrels from which immature ticks have been collected. Following infection of nymphs of D. andersoni with Colorado tick fever virus, the titer of virus drops to very low [illegible] but just after molting to the adult stage, the titer of [illegible] greatly increased.

Although guinea pigs do not succumb to infection with Colorado tick fever virus, it has been possible to demonstrate a viremia persisting for at least 89 days after the animals were infected.

Significance to the Program of the Institute:

The problem of Colorado tick fever has not been adequately defined, although it appears that it is probably the most widespread tick-borne disease in the western United States. The studies thus far carried out have shown that cases of this disease [illegible] severe illness and that this may be a problem of considerable importance. It has been demonstrated that D. andersoni is the principal vector of the disease and that probably a cycle concerning ticks and small rodents is responsible for its continuance in nature.

An intensive program is contemplated in Colorado to determine the incidence of disease and the variation in the clinical picture. This study is to be carried on in cooperation with the State Health Department of Colorado and the Department of Medicine of the University of Colorado Medical School.

Further studies of the peculiar pathology produced by this virus in suckling mice will be continued. The role of certain ticks, such as Dermacentor parumapertus, Dermacentor occidentalis, and Otobius lagophilus, in the maintenance of the virus will be investigated. The role of small rodents and immature ticks in propagating viremia is also being studied. Further emphasis will be given to elaboration of suitable methods for the isolation of virus and for the performance of serological tests.

Consideration is being given to methods of production of a vaccine for the prevention of this disease since it occurs so frequently in rural areas of the western United States.

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PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-101
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$94,000.		$94,000.	2.45	11.75	14.20

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2.70	12.50	15.20	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part C: Honors, Awards, & Publications 15. NIAID-101
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

Eklund, Carl M.

Courtesy appointment as lecturer in virology at Montana State University, Missoula, Montana.

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part A: Project Description Sheet 1. NIAID-102
SERIAL NUMBER

2. NIAID
INSTITUTE OR DIVISION

3. Rocky Mountain Laboratory
LABORATORY, BRANCH, OR DEPARTMENT

4. _____
SECTION OR SERVICE

5. Hamilton, Montana
LOCATION (IF OTHER THAN BETHESDA)

6. Studies on the Encephalitides
PROJECT TITLE

7. Carl M. Eklund
PRINCIPAL INVESTIGATOR

8. Glen M. Kohls, James M. Brennan, William L. Jellison, David B. Lackman, William J. Hadlow, Willy Burgdorfer
OTHER INVESTIGATORS

9. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH. (BY SERIAL NO. (S) IF WITHIN NIH).

10. PROJECT DESCRIPTION

Objectives:

In spite of long continued study, many of the most important aspects of the problems of the encephalitides are still unsettled. We are ignorant of the conditions under which epidemics occur, and data concerning the relationship of epidemics to the abundance of Culex tarsalis and the incidence of infection in these mosquitoes are needed. The factors which result in the occurrence of large populations of this mosquito need to be investigated. Whether or not

these viruses are continually present in an area and are maintained during the winter months in hibernating C. tarsalis or by latent infection in birds and other animal species, or are introduced at irregular intervals by such a mechanism as migrating birds, are questions that must be answered before control methods can be intelligently applied.

Methods Employed:

The methods employed have been previously detailed. Recently, tissue-culture methods have been applied for the detection of neutralizing antibodies, and the hemagglutination-inhibition test and the complement-fixation test have been used for serological diagnosis.

Major Findings:

During 1956, in the Northern Plains area, the incidence of human disease was very low, and only 3 isolations of western equine virus were made from 5,559 C. tarsalis examined. The size of the mosquito collection is a reflection of the small population of these insects. In the Northwestern States, however, the situation was different, there being an outbreak of encephalitis among horses in eastern Washington. This was the first outbreak in approximately 16 years. There was also a recurrence of disease in eastern Oregon and Idaho. Only a small number of human cases were associated with these equine outbreaks. In eastern Washington, C. tarsalis was abundant and in a short period over 11,000 specimens were collected. Western equine virus was isolated 41 times and St. Louis virus 6 times. This relatively low incidence of infection probably accounts for the small number of human cases. In Idaho, approximately 2,500 specimens of C. tarsalis were collected and, among these, western equine virus was isolated on 23 occasions and St. Louis virus on 6. The incidence of infected mosquitoes was approximately four times higher than that in eastern Washington.

St. Louis encephalitis virus was isolated from the brain of a child. This is the first reported isolation of this virus from a human being in the Northwest.

It has been demonstrated that the extrinsic incubation period of St. Louis virus in C. tarsalis is about twice that of the western equine virus and that the donor animal must have a minimal viremia of 10^4 to infect an appreciable number of mosquitoes.

The hemagglutination-inhibition test has been shown to be a valuable tool in the diagnosis of both the western equine and St. Louis types of encephalitis. The tissue-culture neutralization test, employing chick-embryo tissue, has of value in the serological diagnosis of the western equine type of encephalitis.

Continued field studies on the various types of encephalitis are required in order to determine the ecologic factors responsible for the occurrence of these diseases in both a sporadic and an epidemic form. The data which have been accumulated to date indicate that the incidence of disease is associated with a high population level of mosquitoes and with a high incidence of infection among these mosquitoes. It must be admitted that the data at hand do not give information regarding the mechanisms which are responsible for the variations in incidence of the disease.

Proposed Course of the Project:

The problems which at the present time appear to need greatest emphasis are those associated with the possibilities of detecting virus in over-wintering mosquitoes and the possibilities of the occurrence of latent infection in both resident and migrating birds. Studies will be made to determine the ability of C. tarsalis to transmit St. Louis encephalitis virus under varying temperature conditions and to detect viremia due to this virus in common species of birds and mammals in order to determine which of these has a sufficiently high viremia to infect this species of mosquito.

A project to determine the sequelae of infections due to the various arthropod-borne encephalitides is being initiated as a cooperative effort between this Laboratory and certain health and medical agencies in Missouri, Minnesota, and North Dakota.

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PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

Part B: Budget Data 11. NIAID-102
SERIAL NUMBER

12. BUDGET DATA:

	ESTIMATED OBLIGATIONS			MAN YEARS		
	DIRECT	REIMBURSEMENT	TOTAL	PROF	OTHER	TOTAL
FY'57	$70,600.		$70,600.	1.95	8.75	10.70

	BUDGETED POSITIONS			PATIENT DAYS
	PROF	OTHER	TOTAL	
FY'57	2.20	9.00	11.20	

13. BUDGET ACTIVITY:

RESEARCH	[x]	ADMINISTRATION	[ ]
REVIEW & APPROVAL	[ ]	PROFESSIONAL & TECHNICAL ASSISTANCE	[ ]
BIOLOGIC STANDARDS	[ ]		

14. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FY 1957. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Form No. ORP-1 Calendar Year 1956

PUBLIC HEALTH SERVICE - - NATIONAL INSTITUTES OF HEALTH

INDIVIDUAL PROJECT REPORT

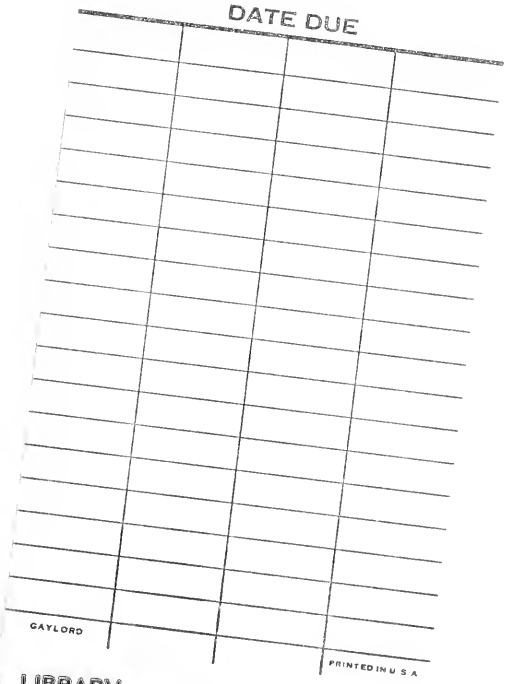
Part C: Honors, Awards, & Publications 15. NIAID-102
SERIAL NUMBER

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1956:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1956:

None



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